ATAZANAVIR AND LOPINAVIR WITH RITONAVIR ALONE OR IN COMBINATION: ANALYSIS OF PHARMACOKINETIC INTERACTION AND PREDICTORS OF DRUG EXPOSURE

G.VISWESWARA RAO M.Pharm (Ph.D)[1], S.PRASANTI M.Pharm[2]

1 Professor in Sri Vani School of Pharmacy, Chevuturu Village,G.Konduru Mandalam, Krishna District, Vijayawada, India,521229.

2Assistant professor in Sri Vani School of Pharmacy, Chevuturu Village,G.Konduru Mandalam, Krishna District, Vijayawada, India,521229.

Objectives

Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Atazanavir and Ritonavir and single method is available for such estimation by RP-HPLC. In view of the need for a suitable RP-HPLC method for routine analysis of Atazanavir and Ritonavir in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Atazanavir and Ritonavir and extend it for their determination in formulation. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of Atazanavir and Ritonavir in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Atazanavir and Ritonavir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and Water (90:10% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 245 nm. The retention time of the Atazanavir and Ritonavir was 2.344, 3.286 ±0.02min respectively. The method produce linear responses in the concentration range of 45-225mg/ml of Ritonavir and 15-75mg/ml of Atazanavir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords : Atazanavir, Ritonavir, RP-HPLC, validation.

1. INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behavior of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analytic. The remainder of the material or sample of which the analytic(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavor, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology.

The primary objective of proposed work is:

- To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Atazanavir and Ritonavir.
- To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Atazanavir and Ritonavir in dosage form.

1.2 Chromatography [2]

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

"] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Types of Chromatography

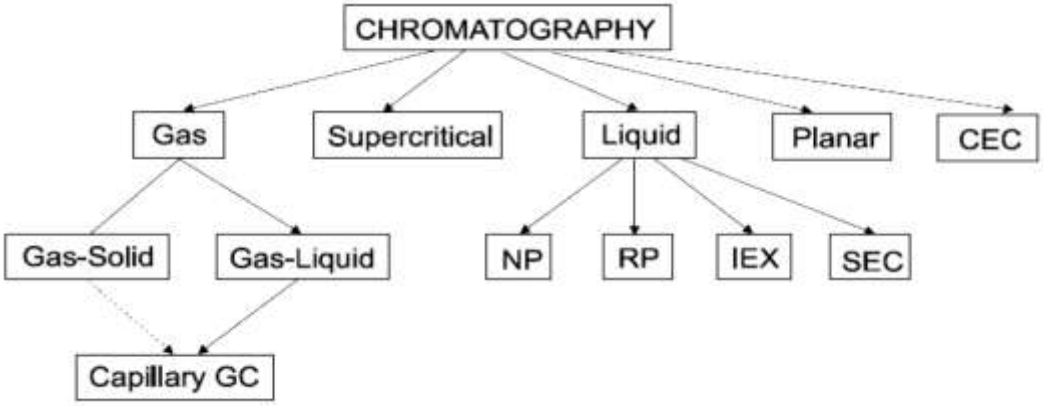

Fig.No.1. Showing flow chart for classification of chromatography

High Performance Liquid Chromatography (HPLC)

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified.

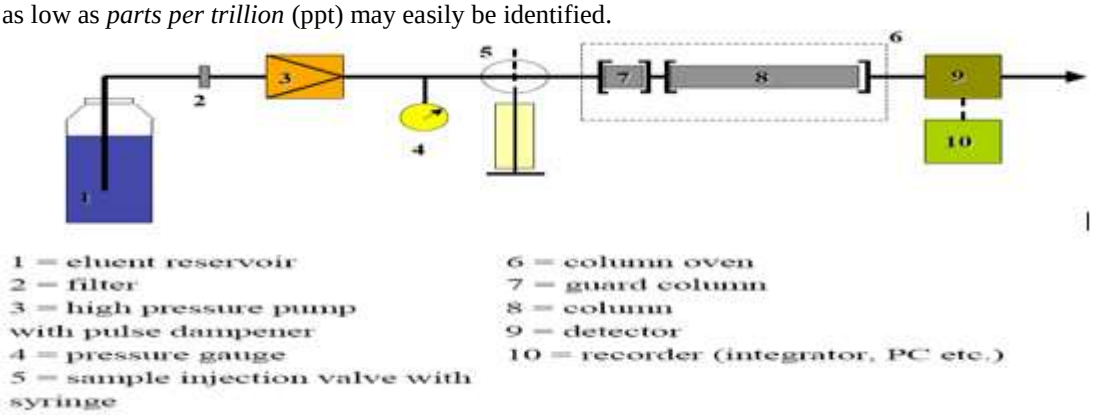

Fig.No.2. High-Performance Liquid Chromatography [HPLC] System

Reversed phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention Time (R_t) is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

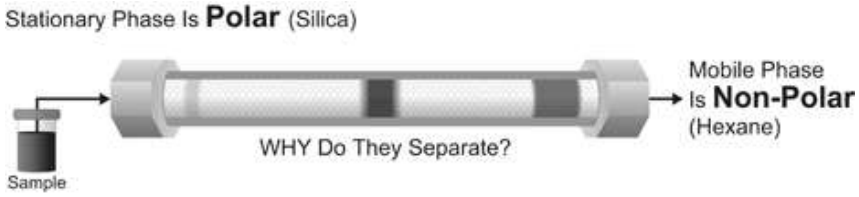

Fig.No.3. Normal-Phase Chromatography

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. The energy released in this process is proportional to the surface tension of the eluent (water: 73 erg/cm², methanol: 22 erg/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding less-polar solvent (MeOH, ACN) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically changing the polarity of the mobile phase during the course of the analysis.

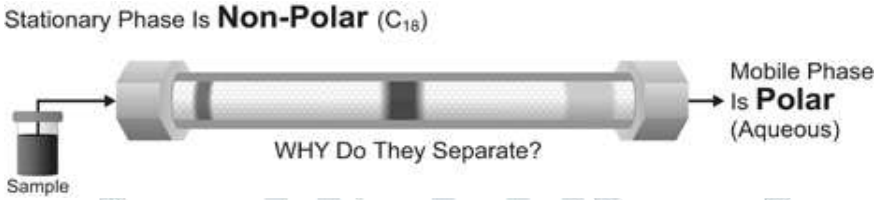

Fig.No.4. Reversed-Phase Chromatography

A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition). The word was coined by Csaba Horvath, who was one of the pioneers of HPLC. The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is the "strong" solvent which rapidly elutes the solutes from the column.

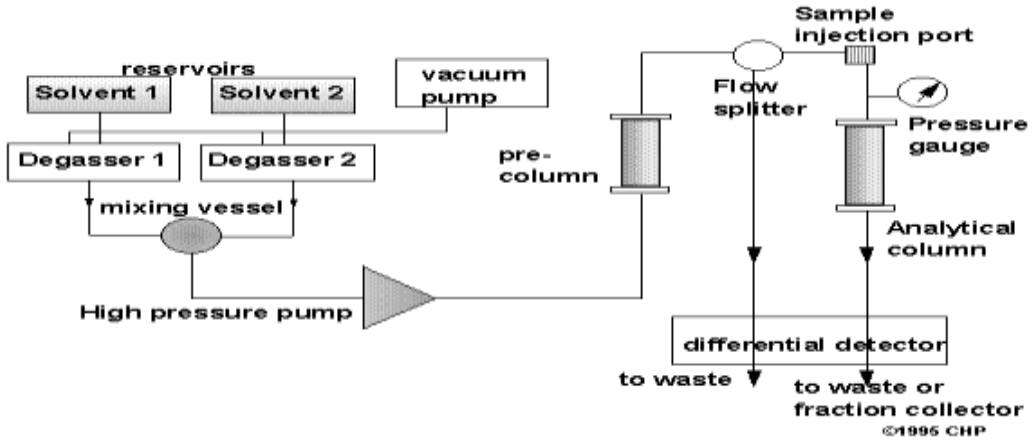

Fig.No.5. High-Pressure-Isocratic System

The most simplified way of explaining the cycle of operation, without taking into account the compressibility of the solvents, is as follows. From the moment when the outlet valve of cylinder a closes and its entrance valve open, the piston in A, moving backwards, sucks the eluent through the inlet check valve and the chamber fills. Meanwhile cylinder B is open and its piston moves forward to force the mobile phase towards the injector and the column. The volume displaced by piston B is half of that available in the chamber of piston A. With chamber A full, the entrance valve of a closes and the corresponding outlet valve opens. Piston a now advances and pushes out the contents of the chamber. Half of this volume is expelled directly towards the column, the other half serves to fill cylinder B as piston B retracts. A pulse absorber is located between the two cylinders (diagram courtesy of Agilent Technologies).

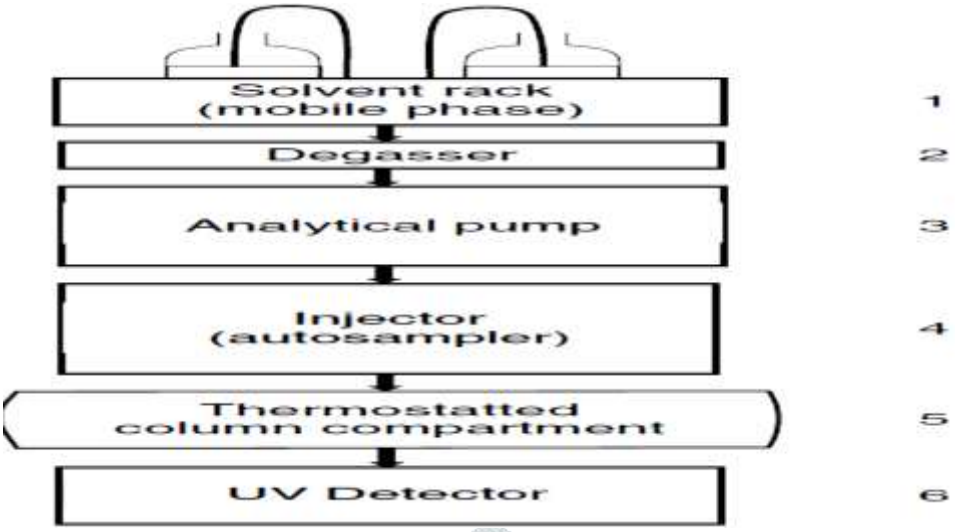

Fig.No.6. Schematic of a modular HPLC instrument

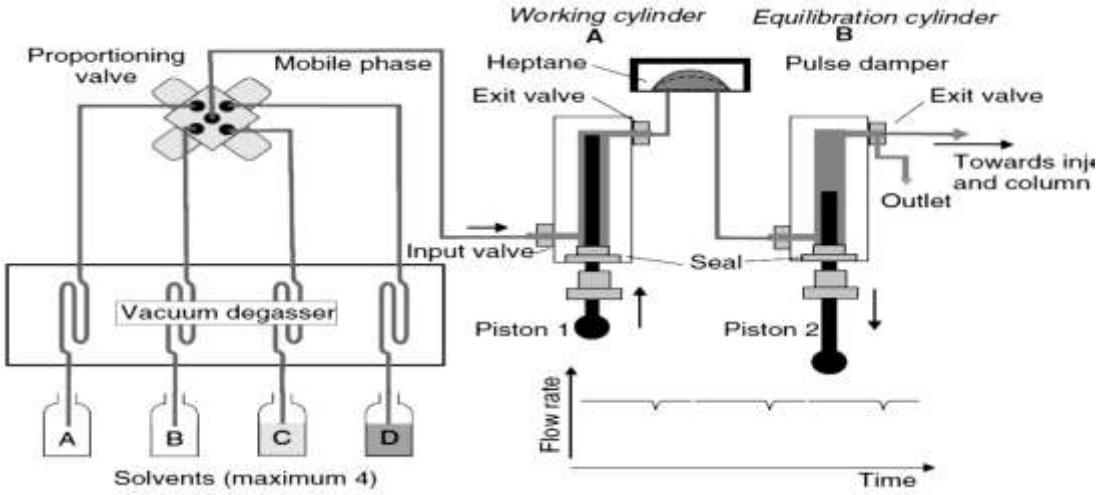

Fig.No.7. Schematic of the dual-headed reciprocating pump

Ultra-Violet (UV) detector

It is divided in to three types they are fixed wavelength, variable wavelength, and diode array detectors.

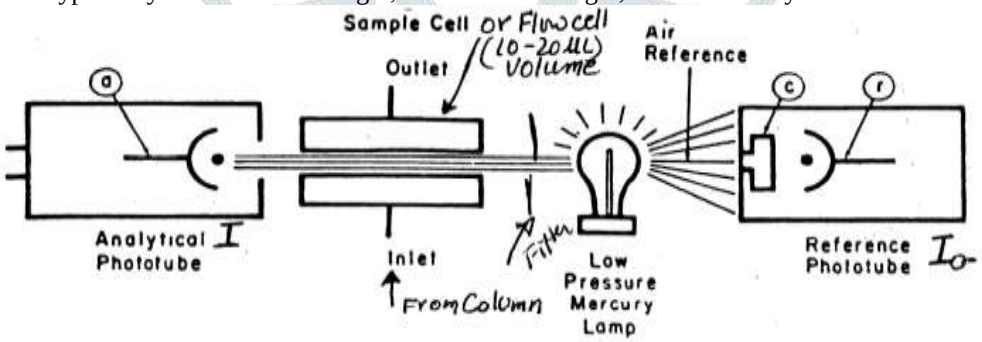

Fig.No.10. Ultra-Violet (UV) Detector

It measures the ability of a sample to absorb light. This can be accomplished at one or several wavelengths

- Fixed wavelength measures at one wavelength, usually 254 nm
- Variable wavelength measures at one wavelength at a time, but can detect over a wide range of wavelengths
- Diode Array measures a spectrum of wavelengths simultaneously
- UV detectors have a sensitivity to approximately 10^{-8} or 10^{-9} gm/ml.

A. Fixed wavelength detectors

a) It is the most common and inexpensive detector. The use of suitable λ is determined by the nature of the light source used.

b) Deuterium lamp can be used over a range of wavelength (covers a continuum of wavelengths), hence covering most of the UV spectral region.

B. Variable wavelength detectors

Provides detection of eluted peak at any selected intensity.

a) It is less sensitive than fixed wavelength but the detection wavelength can be varied.

b) Deuterium source is mostly used because it provides continuum source. This can be combined with a suitable monochromator in dual beam mode.

C. Diode Array detectors (DAD)

- A diode array consists of a number of photosensitive diodes placed side by side and insulated from one another in the form of a multi-layer sandwich.
- The common use of a diode array is to monitor light that has passed through a liquid sensor cell as in a multi-wavelength liquid chromatography detector.
- There are two major advantages of diode array detection:

1. In the first, it allows for the best wavelength to be selected for actual analysis. This is particularly important when no information is available on molar absorptivities at different wavelengths.

2. The second major advantage is related to the problem of peak purity. Often, the peak shape in itself does not reveal that it actually corresponds to two (or even more) components.

HETP is less the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

$$\text{HETP} = \text{Length of column (L)} / N$$

Where

N = plates per meter

RT = retention time of the components.

W = width of the base of the component peak using tangent method.

L = column length in meters

Capacity factor (mass distribution ratio, D_m)

This factor determines the retention of a solute and can be calculated from the chromatogram using the following formula:

$$D_m = \frac{(t_R - t_M)}{t_M}$$

Where,

t_R = retention time of the solute

t_M = retention time of an unretained component

A low D_m value indicates that the peak elutes close to the solvent front, which may compromise selectivity. A minimum D_m value of 1 is recommended for the peak of interest.

Void volume

The volume of the liquid phase in the column is called "*void volume*" (ω). Several other names are also used in the chromatographic literature: "dead volume," "hold-up volume," and sometimes "retention volume of non retained component." In this book we will be using term "void volume."

$$t_R = \frac{V_R}{F}, \qquad t_0 = \frac{V_0}{F}$$

Void time can be interpreted as part of the total analyte retention time that the analyte actually spends in the mobile phase moving through the column, and for the rest of the retention time the analyte sits on the stationary phase surface.

Retention factor

The ratio of the reduced retention volume to the void volume is a widely used dimensionless parameter called *retention factor*, *k*.

$$k = \frac{V_R - V_0}{V_0} = \frac{V_R'}{V_0} = \frac{t_R - t_0}{t_0}$$

For the resolution of a so-called "critical pair" of analytes (two analytes in the mixture that have minimal distance between them compared to all other analytes in the

Mixture), if they have relatively high retention factors ($k\_ > 5$) that their peak widths can be assumed as equal, reduces to

$$R = 2\frac{t_{R.2} - t_{R.1}}{w_2 + w_1}$$

$$R = \frac{t_{R.2} - t_{R.1}}{w}$$

Peak width could be expressed from equation

$$w = \frac{4t_R}{\sqrt{N}}$$

If we select the retention of the second analyte for the calculation of the peak width, then applying equation in to below equation

$$R = \frac{t_{R.2} - t_{R.1}}{t_{R.2}} \cdot \frac{\sqrt{N}}{4}$$

Relatively simple algebraic conversion will bring us to so-called Master Resolution Equation

The efficiency is the measure of the chromatographic band broadening and the number of the theoretical plates (*N*) in the column and is usually calculated using the following equation

$$R = \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{1 + k_2} \cdot \frac{\sqrt{N}}{4}$$

The chemical composition of the sample can provide valuable clues for the best choice of initial conditions for an HPLC separation.

$$N = 16\left(\frac{t_r}{t_w}\right)^2$$

Table No. 1 Table showing special samples and customized conditions

Sample	Requirements
Inorganic ions	Detection is primary problem; use ion chromatography.

Isomers	Some isomers can be separated by reversed-phase HPLC and are then classified as regular samples better separations of isomers are obtained using either (1) normal-phase HPLC or (2) reversed-phase separations with cyclodextrin-silica columns.
Enantiomers	These compounds require "chiral" conditions for their separation.
Biologicals	Several factors make samples of this kind "special": molecular conformation, polar functionality, and a wide range of hydrophobicity.

Optimization of HPLC method

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, asymmetry, capacity factor, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.

The various parameters that include to be optimized during method development are

- Selection of mode of separation.
- Selection of stationary phase.
- Selection of mobile phase.
- Selection of detector.

Selection of mode of separation

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak height and peak tailing factor at 5%. Reproducibility of retention times and capacity factor is important for developing a rugged and repeatable method.

Buffers and buffer capacity

Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most commonly employed buffers are phosphate buffers.

Table No. 7 Table showing buffers and buffer capacity

Buffer	pK_a (25°C)	Maximum Buffer Range	UV Cut-off (nm)
TFA	0.3	--	210 (0.1%)
Phosphate, H_2PO_4 pK_1	2.1	1.1-3.1	< 200
Phosphate, pK_2 HPO_4^{2-}	7.2	6.2-8.2	< 200
Phosphate, pK_3 PO_4^{3-}	12.3	11.3-13.3	< 200
Citrate, pK_1 $C_3H_5O(CO_2H)_2(CO_2-)_1$	3.1	2.1-4.1	230
Citrate, pK_2 $C_3H_5O(CO_2H)_1(CO_2-)_2$	4.7	3.7-5.7	230
Citrate, pK_3 $C_3H_5O(CO_2-)_3$	6.4	4.4-6.4	230
Carbonate, pK_1 HCO_3^{2-}	6.1	5.1-7.1	< 200
Carbonate, pK_2 CO_3^{2-}	10.3	9.3-11.3	> 200
Formate	3.8	2.8-4.8	210 (10nM)

Acetate	4.8	3.8-5.8	210 (10nM.)
Ammonia	9.3	8.3-10.3	200 (10nM)
Borate	9.2	8.2-10.2	N / A
TEA	10.8	9.8-11.8	< 200

Mobile phase composition

Most chromatographic separations can be achieved by choosing the optimum mobile phase composition. This is due to the fact that fairly large amount of selectivity can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions. Most widely used solvents in reverse phase chromatography are methanol and acetonitrile. Experiments should be conducted with mobile phases having buffers with different pH and different organic phases to check for the best separations of analyte peak. A mobile phase which gives separation of analyte peak and which is rugged for variation of both aqueous and organic phase by at least ± 0.2% of the selected mobile phase composition should be used.

Selection of detector

The detector was chosen depending upon some characteristic property of the analyte like UV absorbance, florescence, conductance, oxidation, reduction etc. The characteristics that are to be fulfilled by a detector to be used in HPLC determination are,

- High sensitivity facilitating trace analysis.
- Negligible baseline noise to facilitate lower detection, Low dead volume.

Pharmaceutical ingredients do not absorb all UV light equally, so that selection of detection wavelength is important. An understanding of the UV light absorptive properties of the organic impurities and the active pharmaceutical ingredient is very helpful. For the greatest sensitivity λ_{max} should be used. Ultra violet wavelengths below 200nm should be avoided because detector noise increases in this region. Higher wavelengths give greater selectivity.

EXPERIMENTAL WORK

INSTRUMENTS USED

Table: Instruments used

S.No	Instruments And Glasswares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED:

Table: chemicals used

S.No	Chemical	Brand names
1	Atazanavir	Sura labs
2	Ritonavir	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Atazanavir and Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	38ºc
Column	:	Altima C18 (4.6×150mm, 5µ)
Mobile phase	:	Water: Methanol (10:90% v/v)
Flow rate	:	1ml/min
Wavelength	:	245 nm
Injection volume	:	10 µl
Run time	:	5min

VALIDATION

PREPARATION MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 900ml (90%) of Methanol and 100 ml of Water (10%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Atazanavir and Ritonavir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PRECISION

REPEATABILITY

Preparation of Atazanavir and Ritonavir Product Solution for Precision:

Accurately weigh and transfer 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Atazanavir and Ritonavir and calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Pipette out 0.45ml of Atazanavir and 1.35ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Water: Methanol was taken in the ratio and 15:85, 5:95 instead 10:90, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Atazanavir and Ritonavir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Atazanavir and Ritonavir was freely soluble in ethanol, methanol and sparingly soluble in water.

Water: Methanol (10:90% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Atazanavir and Ritonavir in bulk drug and in Pharmaceutical dosage forms.

RESULTS AND DISCUSSION

Result:

Ritonavir:

=10 × 662.3965/878.3

= 7.5µg/ml

Atazanavir:

=10×16724.53/30145

= 5.5µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ritonavir and Atazanavir. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Ritonavir and Atazanavir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

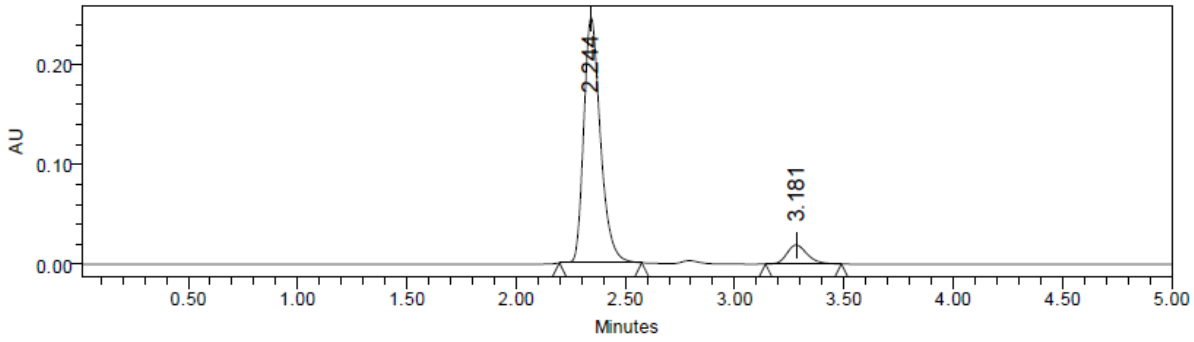

Figure: chromatogram showing less flow of 0.9ml/min

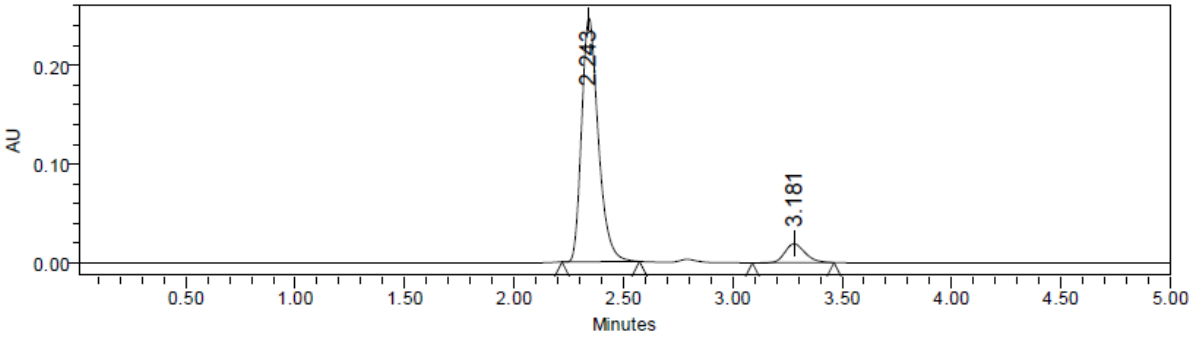

Figure: chromatogram showing more flow of 1.1 ml/min

Variation of mobile phase organic composition

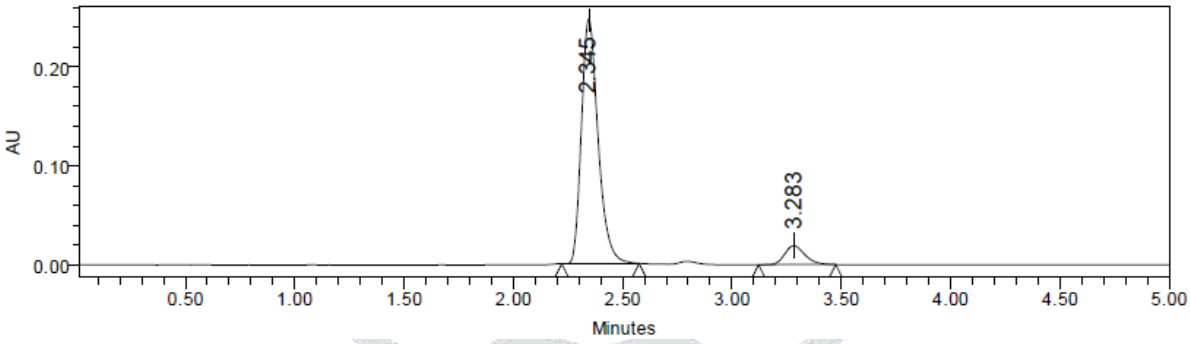

Figure: chromatogram showing less organic composition

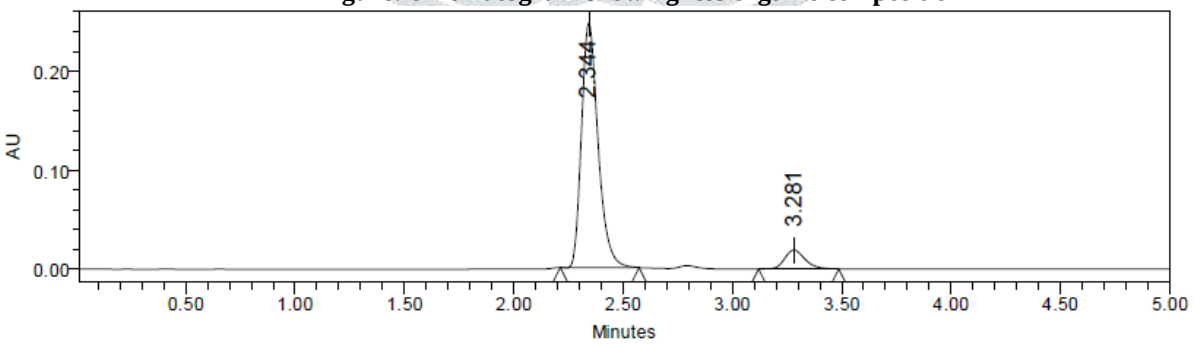

Figure: chromatogram showing more organic composition

Table: Results for Robustness
Ritonavir:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1308495	2.344	5568.2	1.3
Less Flow rate of 0.9 mL/min	1300148	2. 244	5922.2	1.2
More Flow rate of 1.1 mL/min	1306476	2.243	5868.8	1.2
Less organic phase	1304520	2.345	5836.2	1.2
More organic phase	1207845	2.344	5282.6	1.1

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Atazanavir:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	124505	3.286	6098.1	1.2
Less Flow rate of 0.9 mL/min	156550	3.181	5999.1	1.2
More Flow rate of 1.1 mL/min	122702	3.181	5989.2	1.1
Less organic phase	122626	3.278	6387.2	1.1
More organic phase	1207845	3.015	4417	1.1

BIBLIOGRAPHY

[1] Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
[2] A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
[3] Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
[4] Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
[5] Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
[6] Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
[7] Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
[8] Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
[9] Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
[10] Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html