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Simultaneous estimation of atazanavir and ritonavir in combined Tablet dosage form: A Review

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ABSTRACT

In present study describe the development of new method and parameter which will be considered during method development and validation. Article give brief idea for a simple, sensitive, precise and accurate High-performance thin-layer chromatographic method for simultaneous determination of Ritonavir and Atazanavir in their combined tablet dosage form has been developed, validated and used for determination of the compounds in commercial pharmaceutical products. Chromatographic separation will be achieved on different column like C8, C18, etc. used as the stationary phase and different mobile phase.

Keywords: High-performance thin-layer chromatography, Ritonavir, Atazanavir

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1. INTRODUCTION

Atazanavir Sulphate Methyl is a Antiretroviral drug N- [(1S)-1-{ [(2S,3S) - 3 - hydroxy-4- [(2S)-2- [(methoxycarbonyl) amino] -3, 3-dimethyl-N'-{[4-(pyridin-2-yl)phenyl]methyl} butanehydrazido]-1- phenylbutan-2-yl] carbamoyl}-2, 2 - dimethyl propyl] carbamate sulphate is a 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. [1]

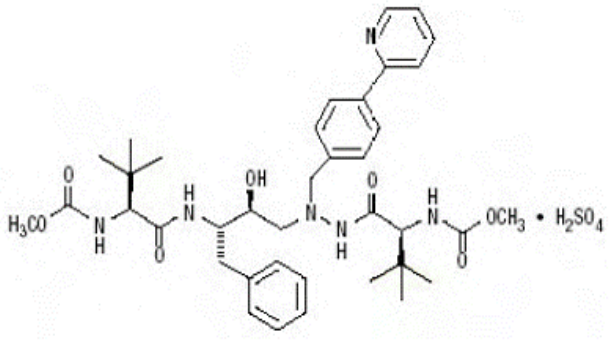

Figure 1: chemical structure of Atazanavir Sulphate

Ritonavir is a Antiretroviral drug 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2{[methyl({[2-(propan-2-yl)-1,3-tiazole-4-yl]methyl})carbamoyl]amino}butane mido]-1,6-diphenyl hexan-2-yl] carbamate. Ritonavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly results. This subsequently results in noninfectious, immature viral particles. [2]

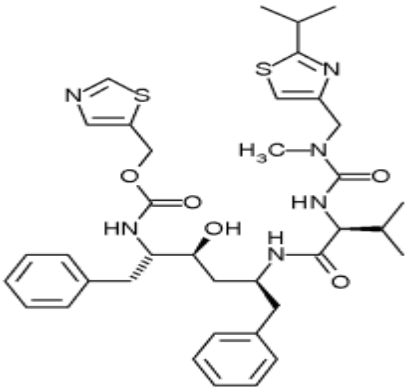

Figure 2: chemical structure of Ritonavir

methods reported for the simultaneous estimation of Atazanavir and Ritonavir gives out information that there are few separate methods reported for the quantitative estimation of Atazanavir sulfate in bulk, pharmaceutical dosage forms and in plasma by HPLC, likewise a very few methods have been reported for the quantitative estimation of Ritonavir by HPLC but till date no method has been reported for the simultaneous quantitative estimation of Atazanavir and Ritonavir by HPLC [3-8]. There is just one spectrophotometric method reported for the simultaneous estimation of Atazanavir sulfate and Ritonavir in tablets. The present developed method was used determine the Atazanavir and Ritonavir present in the formulation and method validated according to the ICH guidelines. [3]

2. INTRODUCTION TO DRUG ANALYSIS

Analytical chemistry is a branch of chemistry that determines the nature and identity of a substance and its composition. In the early twentieth century there were only four accepted branches of chemistry namely, organic chemistry, inorganic chemistry, physical chemistry and biochemistry. Its importance grew, and in the process, absorbed techniques and skills from all other four branches so by the 1950s, analytical chemistry was finally accepted as a branch of chemistry in its own right. [4]

The ability to provide timely, accurate, and reliable data is central to the discovery, development, and manufacture of pharmaceuticals. Analytical data are used to screen potential drug candidates, aid in the development of drug synthesis, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program. [5]

3. INTRODUCTION TO HPLC

HPLC originally referred to the fact that high pressure was needed to generate the flow required for liquid chromatography in packed columns. In the beginning, instrument components only had the capability of generating pressures of 500psi (35 bar). This was called High Pressure Liquid Chromatography (HPLC). [6]

3.1 Instrumentation

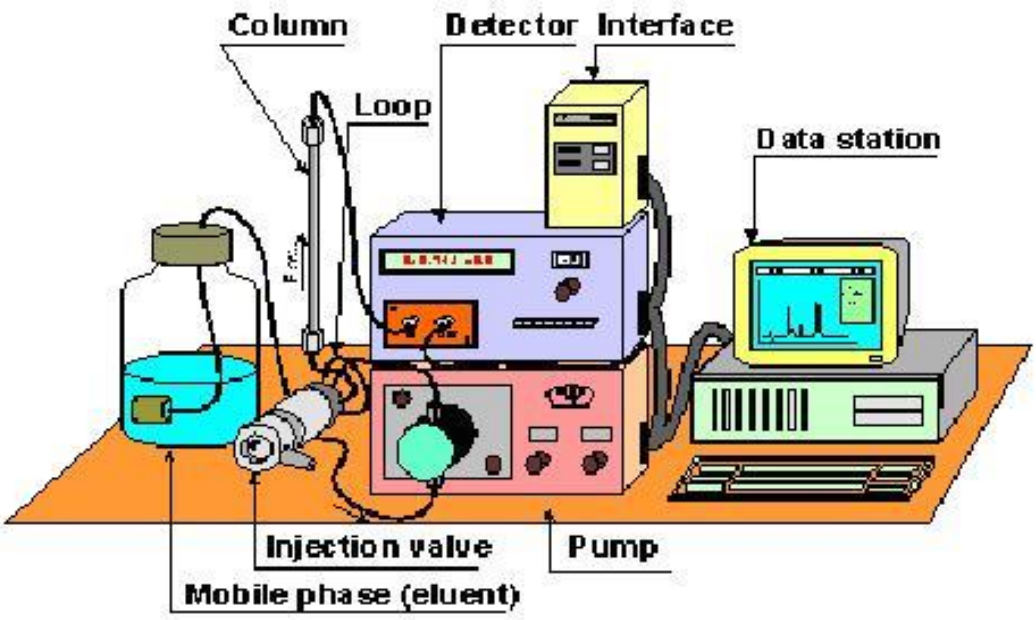

Figure 3: Schematic Diagram of HPLC Instrument

4. INTRODUCTION TO METHOD DEVELOPMENT & VALIDATION

Method development and optimization in liquid chromatography is an attractive field of research for academia and industry. Complex mixtures or samples required systematic method development involving accurate modeling of the retention behavior of the analyte.

4.1 Critical steps for HPLC method development [7]

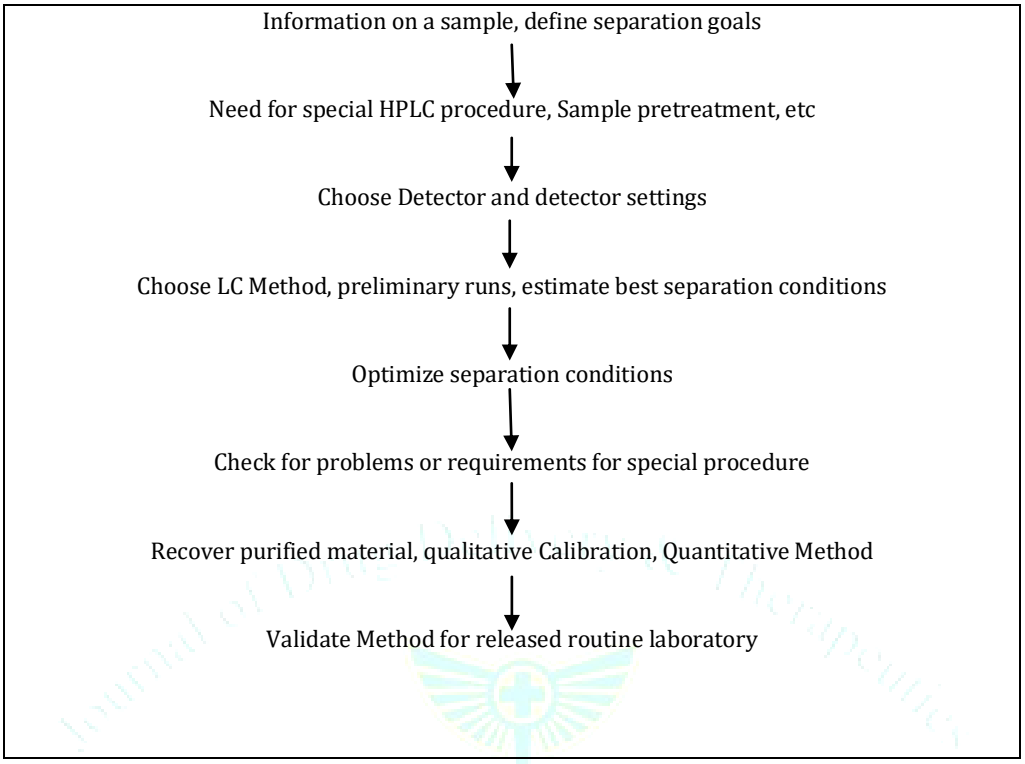

Table 1: Preferred experimental conditions for the initial HPLC run

Separation variable	Initial condition
Column	
Dimensions (length, diameter)	150 × 4.6mm
Particle size	5µm(3.5µm alternatively)
Stationary phase	C8 or C18
Mobile phase	
Solvent A and B	Buffer- Acetonitrile
% of strong solvent	80-100%
Buffer (compound, pH, and conc.)	25mM Potassium phosphate
pH range	2.0 < 3.0
Additives (e.g. Ion pair reagent, amine modifier)	Do not use initially
Flow rate	1.5-2.0 ml/min.
Temperature	350-450 C.
Sample size	
Volume	< 25µl
Weight	< 100µg

4.2 Analytical Method Validation Parameters

Before performing validation of analytical method it is necessary to understand the validation parameters. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows. [8]

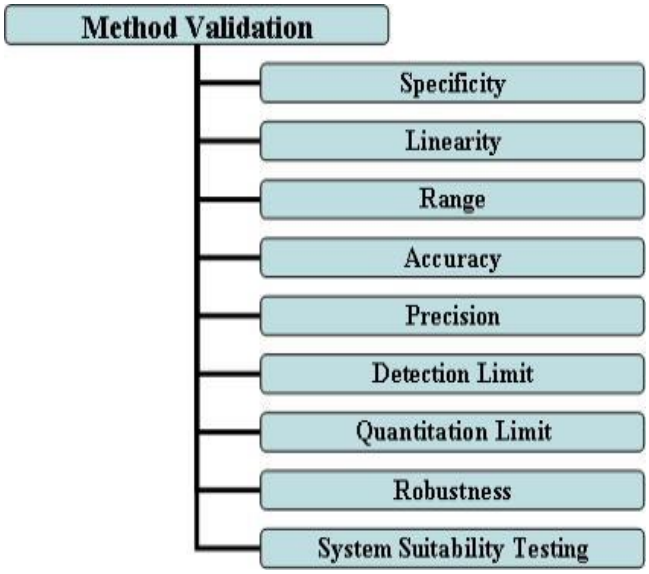

Figure 4: Method Validation Parameters as per USP and ICH

5. PARAMETER FOR METHOD VALIDATION

5.1 Selectivity and Specificity:

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

5.2 Linearity and Range:

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. [9]

5.3 Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. [10]

5.4 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). [11]

5.4.1 **Determination of Repeatability**: Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions like same reagents, equipments, settings and laboratory over a short interval of time. [12]

5.4.2 **Determination of Reproducibility**: Reproducibility means the precision of the procedure when it is carried out under different conditions, usually in different laboratories on separate, putatively identical samples taken from the same homogeneous batch of material. [13]

5.5 Limit of Detection and Limit of Quantization

5.5.1 **Limit of Detection:** The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level. [14]

5.5.2 **Limit of Quantitation**: Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. [15]

5.6 Robustness and Ruggedness

5.6.1 **Robustness:** The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied. [16]

5.6.2 **Ruggedness:** The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. [17]

5.7 Stability of Analytical Solution:

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. [18]

5.8 System Suitability:

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (% RSD, retention time and area for six repetitions) are determined and compared against the specifications set for the method. [19]

Table 2: System Suitability Parameters and Recommendations[20]

Parameters	Recommendation
Capacity Factor (k")	The peak should be well-resolved from other peaks and the void volume, generally K" > 2
Repeatability	RSD ≤ 1% N ≥ 5 is desirable
Relative retention	Not essential as the resolution stated
Resolution (Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipients, degradation products, internal standard, etc.)
Tailing factor (T)	T ≤ 2
Theoretical plates (N)	In general should be > 2000

Table 3: Characteristics to be validated in HPLC [21]

Characteristics	Acceptance Criteria
Accuracy / trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity/ Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient $r^2 > 0.999$
Range	80-120%
Stability	>24 h or > 12 h

5.9 Development of Stability indicating HPLC Method [22]

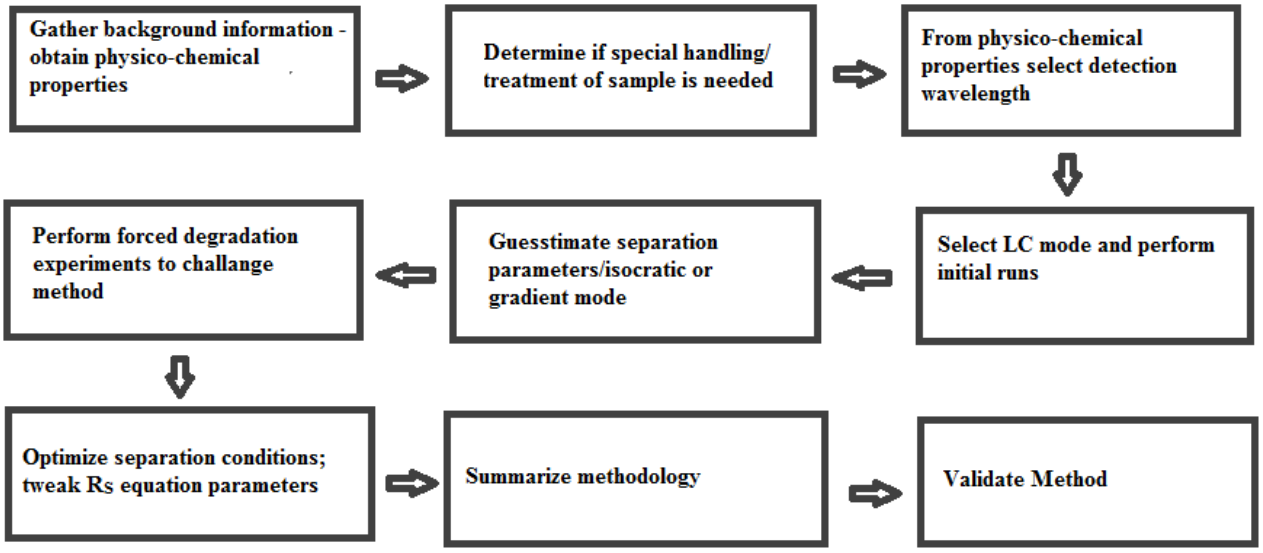

Figure 6: Overview of the Method Development Process

6. CONCLUSION

The results of the validation study indicate that the analytical method developed for the determination of assay is found to be accurate and precise. The percentage RSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained bythis method are in fair agreement. The method is both repeatable and rugged.

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