Analytical Method Development and Validation for the Estimation of Brevaracetam by RP-HPLC Method

Mr. Kartik B. Sakhare[1], Miss. Madhuri M. Theng[2]
[1]M. Pharm Student Gawande College of Pharmacy
[2]Assistance Professor Gawande College of Pharmacy

***Abstract*—The analytical method was developed and validated for estimation of Brivaracetam in bulk and formulation by Reverse Phase High performance liquid chromatography. The separation was carried out on Luna C8 (150 × 4.6mm, 5µm) column. The mobile phase consists of ACN: Water at flow rate 1 ml/min at 208nm. The column temperature was adjusted at 30°C with injection volume 20µl. The retention time of Brivaracetam was 3.1 min. The linearity of the calibration curve was linear over the concentration range 10-50 µg/ml. The developed method was validated according to the International Council for Harmonization (ICH) Guidelines. The developed method was easy, rapid, linear, precise, accurate and consistent. So, the method can be successfully applied for the routine analysis of Brivaracetam in pharmaceutical formulation.**

***Index Terms*—RP-HPLC, Method Validation, Estimation, Chromatogram.**

I. INTRODUCTION

Chromatography is possibly the modern chemist most effective analytical technique available. Its strength derives from its ability to quantitively evaluate, by a single analytical method several individual components present in a mixture. “High performance liquid chromatography (HPLC) is a chromatographic procedure that can isolate a chemical mixture and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. In the area of biological separation and purification, reversed phase chromatography has established both analytical and preparative application. Because of its vast application range, reversed phase chromatography is presently the most extensively utilized separation technology in HPLC. It is believed that in the reverse phase mode, approximately 65 percent (potentially up to 90) percent of All HPLC separations are carried out. “The reasons for this include the reverse phase method simplicity, flexibility and reach as it is capable of handling compounds of a variety of polarities and molecular masses The drug investigations manage in three major ways, viz; creation on an analytical technique for product intermediates for crude materials, active ingredients and substances. Development of analytical techniques for specific medication examination in the presence of excipients, degradation products, and impurities in addition to the identification of the product of degradation, the pathway of degradation, and the extent of degradation when put away under surrounding and accelerated conditions. Development of an insightful technique in an organic framework for nano and semi-micro amounts of medications and their metabolites.(1,2)

Chromatography is a commonly used technique in clinical laboratories for diagnosing inborn carbohydrate, protein and lipid metabolism errors. The parameters quantified are vitamins, hormones, metabolites, tumour markers and drugs in the body fluid in pharmacology, chromatography is used to estimate the purity and pollutants and help discover may new biomolecules, providing insights into disease mechanisms and biomarker discovery. many new biomolecules, providing insights into disease mechanism and biomarker discovery. high pressure liquid chromatography here, the solvent mixture can pass through column containing stationary phases under high atmospheric pressure of 10 pa to 400 pa. [3,4]

1.1 Analytical Methods

Analytical chemistry consists of classical, wet chemical methods and modern analytical techniques. Classical and qualitative methods use separations such as precipitations, extraction and distillation

Identification may be based on differences in colour, odour, melting point, boiling point, solubility, radioactivity or reactivity. Classical quantitative analysis uses mass or volume changes to quantify amount. Instrumental methods may be used to separate samples using chromatography, electrophoresis or field flow fractionation. Then qualitative and quantitative analysis can be performed. Often with the same instrument can separate, identify and quantify an analyte.

Qualitative Technique: It deals with the identification and characterization of substances.

Quantitative Technique: It delivers statistical data concerning the number of specified species in a plan measure of the sample.

1.2 Chemical Method

A chemical method refers to any analytical or experimental approach that uses chemical reactions, reagents, or transformations to detect, identify, separate, or quantify a substance.

Main types of chemical methods

A. Volumetric (Titrimetric) Method

Based on titration, where a solution of known concentration reacts with the analyte

- Acid base titration
- Redox titration
- Complexometric titration

B. Gravimetric Method

Based on measuring the mass of a substance after a chemical reaction

- Precipitation
- Volatilization

C. Calorimetric Method

Use colour changes in chemical reactions to estimate concentration, often with spectrophotometry.

D. Instrumental Method

Instrumental methods are modern analytical technique that use sophisticated instrument to identify, quantify, and study the structure of chemical substances.

- UV visible spectroscopic method
- Infrared Spectroscopy (IR)
- Mass Spectroscopy (MS)
- Atomic Absorption emission Spectroscopy (AAS/AES)
- Nuclear Magnetic Resonance (NMR)

E. Chromatographic Method

- High Performance Liquid Chromatography (HPLC)
- High Performance Thin Layer Chromatography (HPTLC)
- Gas Chromatography (GS)
- Thin Layer Chromatography (TLC)
- Ion Exchange Chromatography
- Electroanalytical Methods
- Potentiometry (pH mater, ion selective electrodes)
- Conductometry
- Polarography

F. Thermal Method

- Thermogravimetric Analysis (TGA)
- Differential Scanning Calorimetry (DSC)
- Differential Thermal Analysis (DTA)

G. Hyphenated Techniques

- GC-MS
- LC-MS
- LC-NMR
- GC-NM

H. Other Method

- Kjeldahl method
- Karl Discher
- Flame Photometry
- ELISA
- Radioimmunoassay
- Differential Scanning Calorimetry
- Thermogravimetric Analysis

1.3 Chromatography

Chromatography is a widely used analytical technique for separating, identifying, and quantifying components of a mixture. Chromatography is based on the principle that different compounds in a mixture distribute themselves between a stationary phase (solid or liquid support) and a mobile phase (liquid or gas) at different rates, leading to separation.

The method was developed by botanist scientist Mikhai Tswett in 1901-1905 in universities of Kazan and Warsaw. He developed the technique and coined the term chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophyll. Since these components separate in bands of different colors (green, orange, and yellow respectively) they directly inspired the name of the technique. New types of chromatography developed during the 1930s and 1940s made the technique useful for many seperation process. (5,6,7,)

A technique used primarily for the separation of the analytes from the mixtures. The samples are distributed between two phases, one of which is the mobile phase, and the other being the stationary phase. Both phases, mobile and stationary, are important for the preparation of analytes. A liquid supported on a solid, a solid or a gel could be the stationary phase and could be packed in a column, spread a layer or disseminated as a film. Vapours (gas) of fluid could be the mobile phase (liquid). Mobile phase is a liquid or gas that carries the samples and propels the compounds through the stationary phase, resulting in separation. By adjusting the composition of the mobile phase, the migration rate can be controlled, leading to efficient separation. The mobile phase can be isocratic or gradient, polar or non polar, based on the nature of the analyte mobile phase is a liquid or gas tat carries the sample and propels the compounds through the stationary phase, resulting in separation By adjusting the composition of the mobile phase, the migration rate can be controlled, leading to efficient separation. The mobile phase can be isocratic or gradient, polar or non-polar, based on the nature of the analyte. Stationary phase is a crucial components of chromatography that interact with analyte as they pass through, leading to separation. Solid, liquid, or gas can be held over a supporting medium. The stationary phase interact with various mixture components based on the polarity, affinity, size and charge. Thus different analytes to differential retention times and elution profiles.(8,9,10)

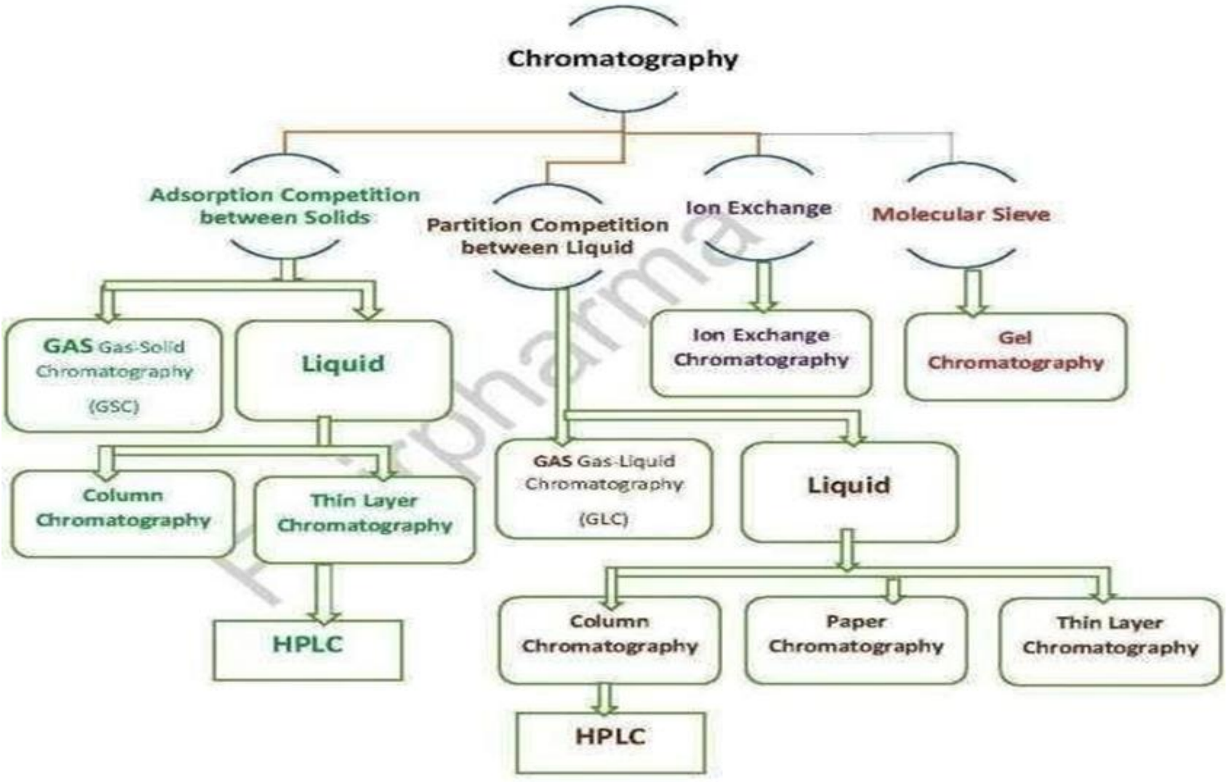

Figure 01 : - Types of Chromatography

1.4 Based on the stationary and mobile phase used

A. Reverse Phase HPLC

Reversed phase HPLC (RP-HPLC) is the most widespread mode of chromatography. It has a non-polar stationary phase and an aqueous, moderately polar mobile phase. In the reversed phase methods, the substances are retained in the system the more hydrophobic they are.

There are also polymeric hydrophobic particles that serve as stationary phases, when solutions at extreme pH are needed, or hybrid silica, polymerized with organic substances. The longer the hydrocarbon ligand on the stationary phase, the longer the sample components can be retained.

B. Normal Phase

Normal phase chromatography was one of the first kind of HPLC that chemists developed. But has decreased in use over the last decades. Also known as normal-phase HPLC (NP- HPLC), this method separates analytes based on their affinity for a polar stationary surface such as silica; hence it is based on analyte ability to engage in polar interactions (such as hydrogen- bonding or dipole-dipole types of interactions) with the sorbent surface. The use of more polar solvents in the mobile phase will decreased the retention time of analytes whereas more hydrophobic solvents tend to induce slower elution.

1.5. Based on Technique of Elution

A. Isocratic Separation

Isocratic separation is a type of liquid chromatography separation where the mobile phase compositions remain Compounds are separated based on their relative interactions with the stationary phase vs. the fixed mobile phase. Constant throughout the entire run.

B. Gradient Separation

Gradient separation is a chromatographic technique where the composition of the mobile phase is gradually changed during the separation process to improve resolution of compounds with different polarities. Gradient separation is a chromatographic technique where the mobile phase compositions varies over time during the separation process. This approach is crucial for analysing samples with components that have different retentivity as it enhances the resolution of complex mixtures. By adjusting the mobile phase dynamically

gradient separation improves the separation efficiency and clarity of the results in chromatography.

1.6. Based on the operational scale

A. Analytical HPLC

Analytical HPLC (High- Performance Liquid Chromatography) is a separation technique used to identify, quantify, and assess the purity of compounds in a mixture. Unlike preparative HPLC which focuses on isolating large amount of material, analytical HPLC is primarily concerned with precise measurement and analysis at microgram-to-milligram levels.

B. Preparative HPLC

Preparative HPLC (High Performance Liquid Chromatography) is a chromatographic technique used for the isolation and purification of large quantities of compounds, unlike analytical HPLC which focuses on separation and quantification.

1.7. Based on the type of Analysis

A. Qualitative Analysis

In most cases, identification of a sample component is performed by comparing its retention time with that in a standard sample. If a complex chromatogram with many peaks is obtained or if the retention time of the target component differs between the standard and the actual sample, the target component is identify by adding the standard sample to the unknown sample.

B. Quantitative Analysis

There are two methods of quantifications the external and internal standard methods. Both of which are performed using a calibration curve. The external standard methods creates a calibrations curve for a standard sample and unknown are quantified using the calibration(11,12,13)

1.8. HPLC Instrumentation

HPLC (High performance Liquid Chromatograph) instrumentation consists of several key components that work together to separate, identify and quantify analytes in a mixture. Solvent reservoir, pump system, sample injector, column, detector, data system, fraction collector.

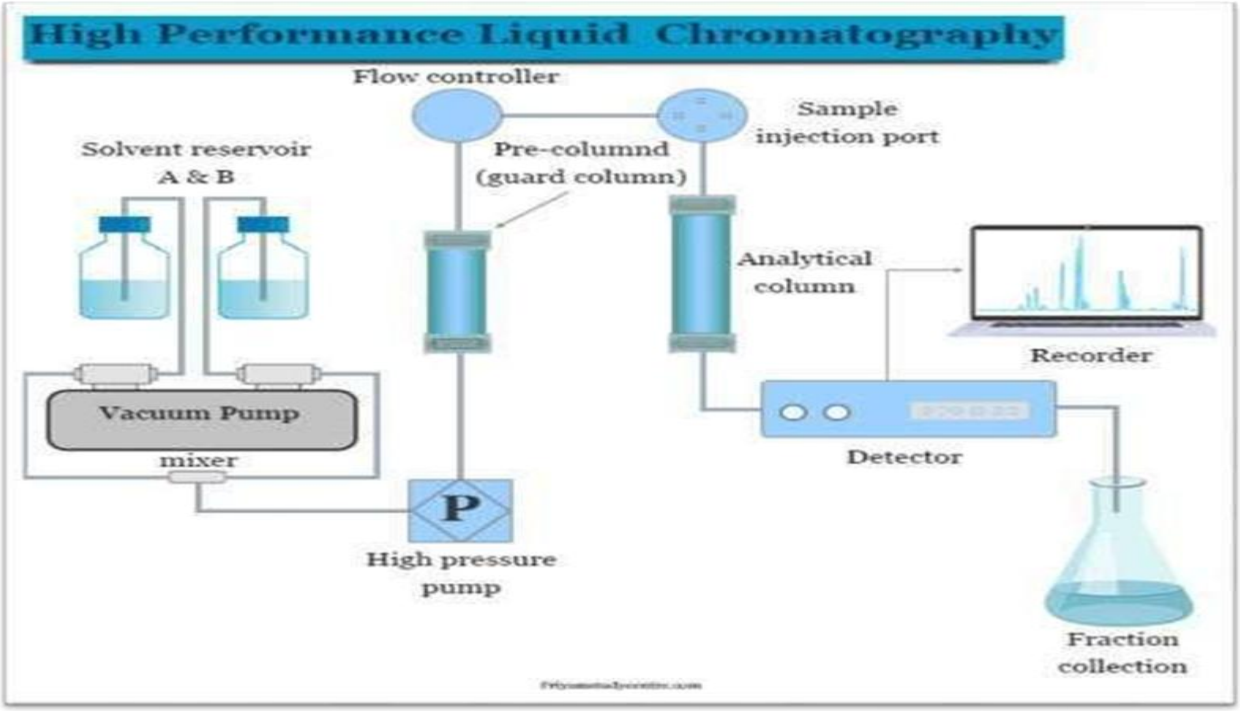

Figure 02 :- A Diagram of HPLC System

1.9. Mobile Solvent Reservoir

Any clean inert container may be utilized as a mobile-phase reservoir. It typically holds 0.5- 2 Liters of solvent and should have a cover that permits the input line of the tube to feed the solvent delivery system to the mobile process. Glass is often employed in microscopic molecular analysis to prevent plastic components from leaching into the eluent and interfering with the experiment. In certain mobile phase tanks, the labelling is on the surface for an accurate understanding of the number of solvents. The cap is also used to remove dust, minimize solvent evaporation, allow the bottle to be pressurized, give ports for additional inlet lines and save.

Figure 03 :- A Diagram of HPLC Reservoir

1.10. Sample Injector Device

A sample injector is a crucial device in HPLC systems that introduces a small specified volume of a sample into mobile phase steam for analysis by controlling the volume and pressure of the introduced liquid sample. Its efficiency in sample introduction is fundamental for obtaining reliable analytical results in liquid chromatography processes.

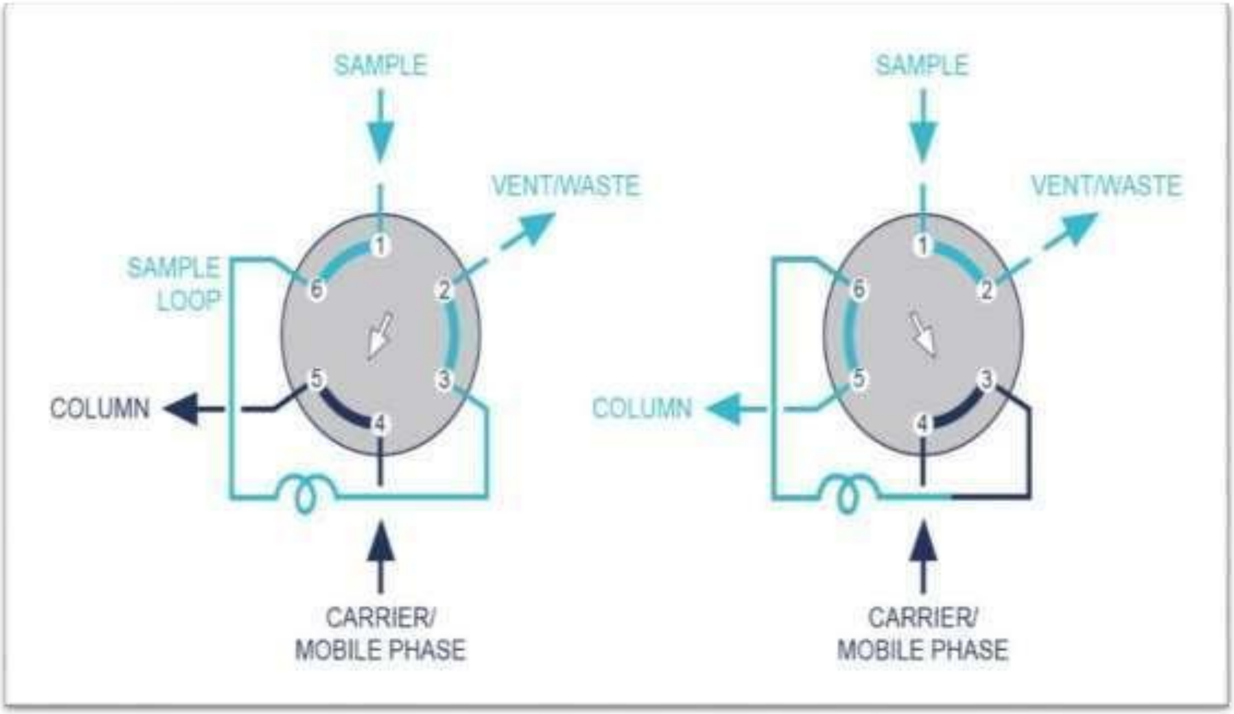

Figure 04 :- A Diagram of Sample Injector Device

1.11. Solvent Delivery System

The pump purpose is to provide accurate, precise, repeatable, continuous and pulse-free mobile phase flow to the column while also maintaining uniform pressure (500 to 20,000psi).

There are presently three kinds of

A. Constant Pressure Pump
B. Syringe pump or displacement pump
C. Constant flow pump

Constant flow pump is the most common pump used in HPLC devices. A single reciprocating piston pump underpins the great majority of high pressure. Industrial pumps on the market today. The piston is pushed in and out of the solvent chamber of the pump head which has a capacity of 10-100 L. The piston enters the solvent chamber during the delivery stoke, causing friction. The mobile phase may flow to the column due to the high pressure. The intake control valve opens and outlet control valve shuts as a result of the low pressure.

1.12. Column

The column is the main part of the HPLC system, where the separation happens. HPLC columns are primarily made from stainless steel to deal the high-pressure (5000psi). The column is also made up of other materials such as heavy walled glass, titanium, and plastic. Generally the guard column and analytical column are two types of columns used in HPLC.

A. Guard Column

Guard column has exceptionally low adsorbent concentrations which extends the columns life the removal of particulate matter and other substances. If any typically serves as a prefilter. The guard column is made up of the same material as the analytical column. The guard column does not separate anything but it serves as a security guard to protect the analytical column from impurities or other outside obstacles.

B. Analytical Column

The column length ranges from 50 mm to 300 mm and its inner diameter ranges from 2 mm to 5 mm. They are usually filled with a fixed step with particle size ranging from 3 m to 10
m. Stationary processes 1000 mg has a surface area of 100-860 sq. m. With a 400 sq. m. average. Columns with internal diameter of less than 2 mm are typically proposed as microbore segments regularly. The new form of material used for the development of columns is PEEK (Polyether Ether Ketone) various types of internal coating material for columns (stationary phase) are used. (14,15,16)

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