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**Research**

# Reverse phase-hplc method development and validation for the quantitative estimation of brivaracetam in bulk form and marketed pharmaceutical dosage form

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## Abstract

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Brivaracetam in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (pH-3.6 adjusted with OPA) (35:65%) v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Brivaracetam was found to be 2.286min. The calibration plot was linear over the concentration range of 6–14µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Brivaracetam in bulk and marketed pharmaceutical dosage form dosage form.

**Keywords:** Brivaracetam, RP-HPLC, Validation, Accuracy, ICH Guidelines.

## INTRODUCTION

### Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

**Introduction to chromatography, its types and classification Chromatography**

Chromatography is a set of techniques in which separation of chemical substances takes place quantitatively as well as qualitatively.

**Terminology used in Chromatography Mobile Phase:** In chromatography the substance which is introduced with or along with the sample and causes elution of the contents of the sample. It may be liquid or gas.

**Stationary phase**: Stationary phase of the chromatographic system refers to that part which is present before the introduction of sample or solute in the column (as in column chromatography) or on a solid support (as in paper or similar chromatography). It may be liquid or solid.

**Eluent:** The substance which separates the components of the mixture in chromatographic technique. Eluent is that part which brings separation when the solution is passed either from the column or from the solid support.

**Eluate:** The substance which is separated as a individual component of the mixture is called eluate.

**Important types of Chromatographic Techniques**

Following are some important types of Chromatographic separation techniques. They are defined thoroughly by explaining their general principle, application, and a brief outline of their instrumentation for a complete understanding. Following are some commonly utilized types of techniques:

i. Gas Chromatography
ii. High Pressure Liquid Chromatography
iii. Supercritical fluid Chromatography
iv. Gel Exclusion Chromatography.

**High Pressure Liquid Chromatography (HPLC)**

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed, and the solvent, or solvents used. As the sample passes through the column it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes.

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography.

Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

## MATERIALS AND METHODS

Brivaracetam (Pure) Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

## HPLC METHOD DEVELOPMENT

### Trails

### Preparation of standard solution

Accurately weigh and transfer 10 mg of Brivaracetam 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.

Further pipette 0.1ml of the above Brivaracetam stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

### 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.

### Mobile Phase Optimization

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

### Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5μm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**Optimized chromatographic conditions**:

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : Ambient
Column : Symmetry ODS C18 (4.6×250mm, 5μm)
Mobile phase : Methanol: Phosphate Buffer pH-3.6 (35:65% v/v)
Flow rate : 1ml/min
Wavelength : 235nm
Injection volume : 10μl
Run time : 8minutes

### Validation

### Preparation of buffer and mobile phase

### Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

### Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 μ filter under vacuum filtration.

### Diluent Preparation:

The Mobile phase was used as the diluent.

## RESULTS AND DISCUSSION

Mobile phase ratio : Methanol: Phosphate Buffer (35:65%) v/v
Column : Symmetry ODS C18 (4.6×250mm, 5μm)
Column temperature : Ambient
Wavelength : 235nm
Flow rate : 1ml/min
Injection volume : 10μl
Run time : 8min

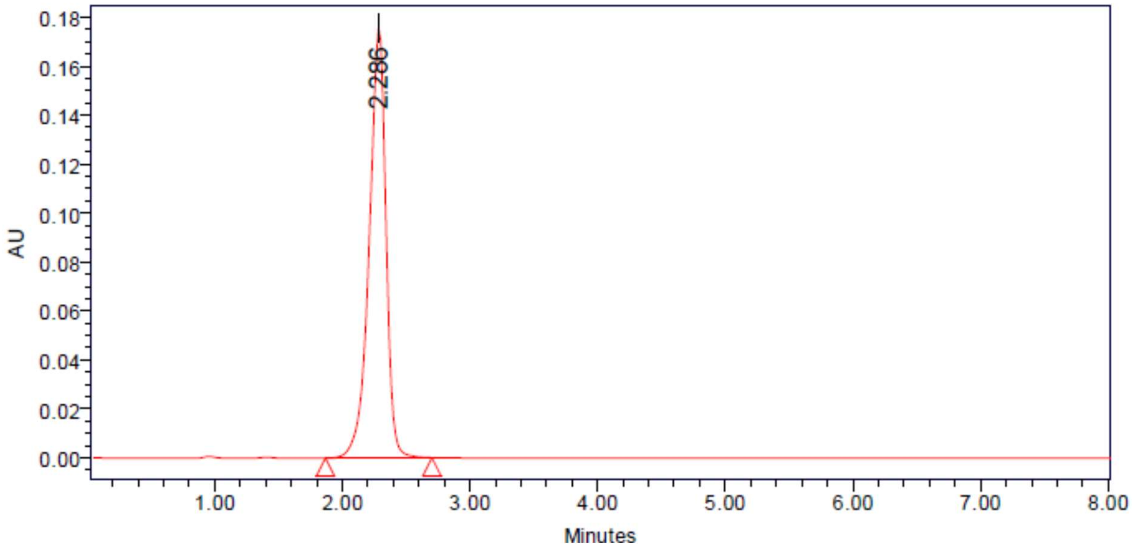

**Fig 1: Optimized Chromatogram (Standard)**

**Table 1: Optimized Chromatogram (Standard)**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.286 | 1658242 | 185421 | 1.24 | 6569 |

In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So, it is an optimized chromatogram.

**Optimized Chromatogram (Sample)**

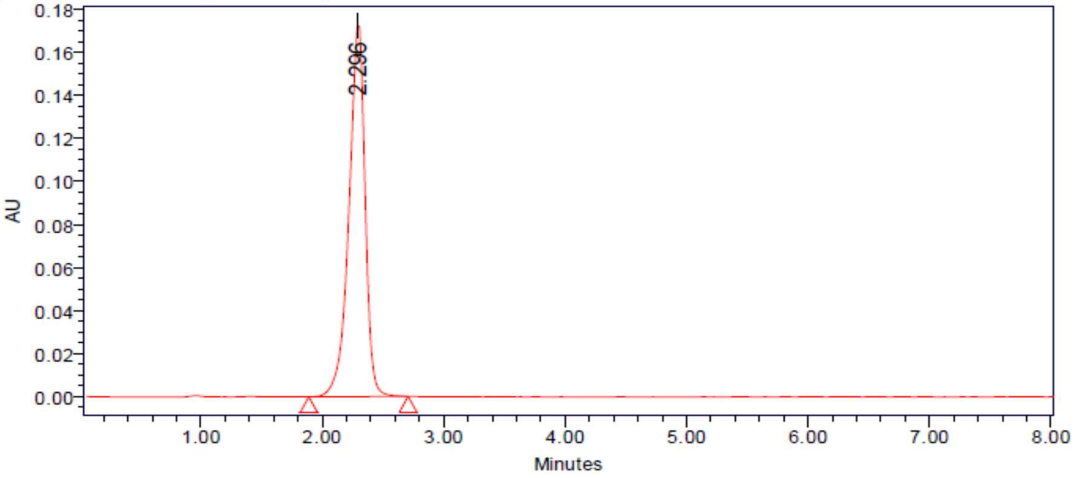

**Fig 2: Optimized Chromatogram (Sample)**

**Table 2: Optimized Chromatogram (Sample)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.296 | 1689654 | 185231 | 1.28 | 6659 |

- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be less than 2.*

It was found from above data that all the system suitability parameters for developed method were within the limit.

### Assay (Standard)

**Table 3: Results of system suitability for Brivaracetam**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.277 | 1652847 | 185647 | 6589 | 1.24 |
| 2 | Brivaracetam | 2.277 | 1653658 | 186254 | 6587 | 1.26 |
| 3 | Brivaracetam | 2.267 | 1654521 | 185475 | 6584 | 1.28 |
| 4 | Brivaracetam | 2.265 | 1653564 | 186594 | 6582 | 1.29 |
| 5 | Brivaracetam | 2.277 | 1658745 | 185684 | 6895 | 1.24 |
| **Mean** | | | 1654667 | | | |
| **Std. Dev.** | | | 2355.764 | | | |
| **% RSD** | | | 0.142371 | | | |

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

**Table 4: Peak results for Assay sample**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Brivaraceta | 2.246 | 1645879 | 184574 | 0.85 | 6458 | 1 |
| 2 | Brivaraceta | 2.246 | 1645875 | 183598 | 0.86 | 6584 | 2 |
| 3 | Brivaraceta | 2.246 | 1658423 | 185472 | 0.85 | 6457 | 3 |

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

The % purity of Brivaracetam in pharmaceutical dosage form was found to be 99.86%.

### Linearity

**Table 5: Data for Linearity of Brivaracetam**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 6 | 1078475 |
| 8 | 1461129 |
| 10 | 1808358 |
| 12 | 2211573 |
| 14 | 2593778 |

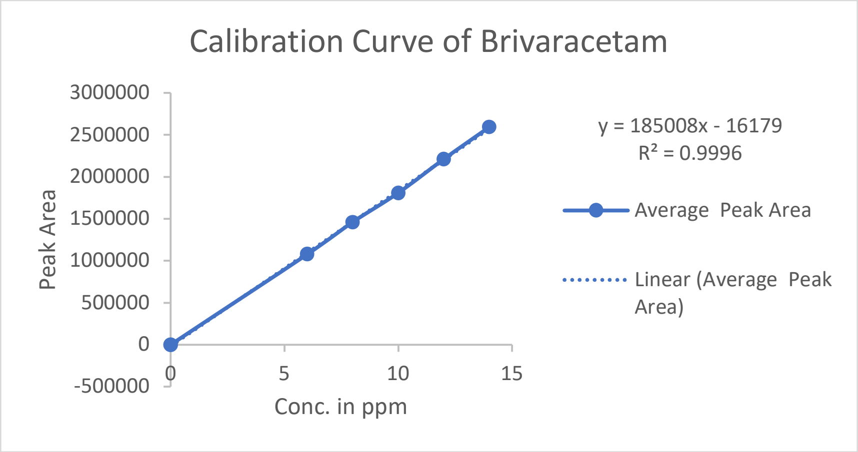

**Fig 3: Linearity Curve of Brivaracetam**

### Repeatability

**Table 6: Results of repeatability for Brivaracetam**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.293 | 1658954 | 186958 | 1.26 | 6785 |
| 2 | Brivaracetam | 2.276 | 1658745 | 187548 | 1.27 | 6854 |
| 3 | Brivaracetam | 2.286 | 1659865 | 189854 | 1.26 | 6852 |
| 4 | Brivaracetam | 2.277 | 1653254 | 186985 | 1.25 | 6784 |
| 5 | Brivaracetam | 2.280 | 1654781 | 189542 | 1.24 | 6895 |
| **Mean** | | | 1657120 | | | |
| **Std.dev** | | | 2913.592 | | | |
| **%RSD** | | | 0.175823 | | | |

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

### Intermediate precision

**Table 7: Results of Intermediate precision for Brivaracetam**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.274 | 1678541 | 186589 | 6587 | 1.26 |
| 2 | Brivaracetam | 2.258 | 1685985 | 186598 | 6321 | 1.26 |
| 3 | Brivaracetam | 2.267 | 1685745 | 186985 | 6385 | 1.25 |
| 4 | Brivaracetam | 2.270 | 1685987 | 187854 | 6580 | 1.26 |
| 5 | Brivaracetam | 2.264 | 1698526 | 187549 | 6721 | 1.27 |
| 6 | Brivaracetam | 2.265 | 1685943 | 186598 | 6637 | 1.26 |
| **Mean** | | | 1686788 | | | |
| **Std. Dev** | | | 6463.466 | | | |
| **% RSD** | | | 0.383182 | | | |

- *%RSD of Six different sample solutions should not more than 2.*

**Table 8: Results of Intermediate precision Analyst 2 for Brivaracetam**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Brivaracetam | 2.277 | 1665847 | 167481 | 6854 | 1.25 |
| 2 | Brivaracetam | 2.255 | 1658989 | 167854 | 6785 | 1.26 |
| 3 | Brivaracetam | 2.265 | 1659845 | 167895 | 6854 | 1.24 |
| 4 | Brivaracetam | 2.255 | 1665964 | 167854 | 6895 | 1.26 |
| 5 | Brivaracetam | 2.253 | 1659863 | 168585 | 6459 | 1.25 |
| 6 | Brivaracetam | 2.252 | 1665986 | 167859 | 6456 | 1.26 |
| **Mean** | | | 1662749 | | | |
| **Std. Dev.** | | | 3501.766 | | | |
| **% RSD** | | | 0.210601 | | | |

- *%RSD of Six different sample solutions should not more than 2.*

### Accuracy

**Table 9: The accuracy results for Brivaracetam**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 109068.3 | 5 | 5.021 | 100.420% | 100.72% |
| 100% | 202187 | 10 | 10.054 | 100.540% | |
| 150% | 297032.3 | 15 | 15.181 | 101.206% | |

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

**Robustness**

**Table 10: Results for Robustness**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 1658242 | 2.286 | 6569 | 1.24 |
| Less Flow rate of 0.9 mL/min | 1854215 | 2.458 | 6865 | 1.35 |
| More Flow rate of 1.1 mL/min | 1758468 | 2.032 | 6254 | 1.32 |

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

## CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Brivaracetam 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. Brivaracetam was freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and octanol, and practically insoluble in water. Methanol: Phosphate Buffer (pH-3.6) (35:65%) 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 Brivaracetam in bulk drug and in Pharmaceutical dosage forms.

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