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Development and Validation of a Stability-Indicating High-Performance Liquid Chromatography (HPLC) Method for the Determination of Brivaracetam

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ABSTRACT:

Brivaracetam (BRV) is a one of the powerful drug used to control seizures. It belongs to a group of drugs called levetiracetam analogs. This drug shows its action by attacking to a protein which is found in brain cells called synaptic vesicle protein 2A (SV2A). It is must to keep the drug clean and stable form in the different forms used in medicine so it works effectively for patients. This review examines at recent work on methods to test how stable Brivaracetam is, including techniques such as High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography (UPLC). It also examines research on how the drug changes when it exposed to stressful conditions, how to find impurities, and how to follow the ICH guidelines to make sure these testing methods are dependable.

Keywords: Brivaracetam, Anti-epileptic, HPLC, Stability-indicating method, Forced degradation, Method validation

1. INTRODUCTION

Brivaracetam is a drug belongs to the category of antiepileptic drug that has become standard drug in treating epilepsy because it works effectively and binds more strongly to its target than other older drugs such as levetiracetam [3]. Chemically, it is known as (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl]butamide. It have ability to reduce seizures due to its well attachment to a brain protein called Synaptic Vesicle Protein 2A (SV2A), which helps to release brain chemicals [3]. Since Brivaracetam works by interacting with SV2A, it is important to keep the structure of drug during its manufacturing and storage to make sure it remains effective. During its production and storage, Brivaracetam can change chemically and can develop impurities. These impurities either from the drug manufacturing process or from solvents which are used during the crystallization process [4]. The molecule has two centers that can twist in different ways, which leads to its different forms called isomers. Only one isomer (2S, 4R) of Brivaracetam is active in treating seizures, so it is must to control the presence of all other isomers to ensure the drug works properly [6]. Therefore, it is necessary to develop methods that can tell Brivaracetam apart from its isomers and other unwanted substances, impurities [10]. In quality control for drugs, it is important to create a Stability-Indicating Method, as recommended by ICH Q1A (R2) guidelines [7]. A Stability-Indicating Method is a method of evaluation that can accurately measure how much of the active drug is present and is not affected by broken down parts, other substances or impurities. The stability of Brivaracetam can be affected by many factors, involving moisture, which causes the drug to break down in water and oxygen, which results in oxidation; heat, which results in thermal breakdown; and light, which results in photo degradation [1, 5]. Stress testing or forced degradation studies, helps to find out how the drug breaks down by treating it to extreme conditions in a controlled manner [4, 12]. Studies on Brivaracetam have also aimed n how it is absorbed, spread through the body, broken down and removed [9]. Validated methods for measuring Brivaracetam levels in blood and other body samples are now available, helping physicians to track how much medication is in a patient's system [9]. In addition, new initiatives towards "Green Chemistry" have contributed to the development of more environment friendly HPLC methods that involves less harmful solvents and more efficient testing procedures to analyze Brivaracetam and other brain related medications [2].

2. SCOPE

- This work recognizes the chemical composition, physical and chemical traits of Brivaracetam, and how it stays stable when it is its raw form and when it is made into medicine [1, 9, 2].

- This work evaluates and compares different ways of testing Brivaracetam, mainly focusing on RP-HPLC and UPLC methods that can find any modifications in the drug as time passes [5, 4, 15, 20].

- This review highlights the testing method for Brivaracetam that follows ICH Q2 (R1) guidelines, checking parameters such as linearity, accuracy, precision, and sensitivity [11, 8, 10].

- This article explores that how the Brivaracetam breaks down under various extreme conditions such as acid, base, oxidation, heat, and light, as per ICH guidelines [12, 18, 1].

3. CURRENT PERSPECTIVES

- As Brivaracetam breaks down in large amount when it exposed to oxidative and alkaline conditions, there is a need for testing methods that can clearly show these changes [18, 1].

- Following ICH guidelines means using analyzed and reliable methods for regular quality checks of Brivaracetam. [12, 11, 13].

- Methods such as UPLC and LC-MS are required to find and recognize small amounts of impurities and broken down parts of the drug [4, 16, 10].

- Techniques that use less amount of solvent are important to follow current rules and environmental standards [2, 17].

4. PHARMACOLOGICAL PROFILE OF BRIVARACETAM[3]

Brivaracetam is a drug that binds more strongly and specifically to a protein called Synaptic Vesicle Protein 2A, or SV2A. SV2A is found in the membranes of small bags inside nerve cells which hold neurotransmitters. This protein helps to control how these bags prepare and attach with cell membrane, which is must for sending signals in the brain. Research has found that Brivaracetam binds to SV2A very strongly than other analog called levetiracetam. It binds about 15 to 30 times better. This strong attachment results in the drug's effective function even when it is used in smaller amounts and starts working faster. The ability of Brivaracetam to decrease seizures depends on how much portion of SV2A sites it binds in brain, as shown in studies by using various animal models and human tissue.

5. CHEMICAL STRUCTURE AND PROPERTIES OF BRIVARACETAM

The analytical behavior and stability of Brivaracetam is depend on its molecular structure and its chemical composition. To understand these properties is important for choosing the most suitable analytical conditions, selecting the appropriate column type, and determining how the drug may degrade [1, 15].

Molecular Structure: Brivaracetam is same as levetiracetam but it contains a propyl group instead of methyl group. Its IUPAC name is (2S)-2[(4R)-2-propylpyrrolidin-1-yl]butanamide. Its molecular formula is $C_{11}H_{20}N_2O_2$ [3, 20].

The Brivaracetam molecule consists of three main components:

- Pyrrolidine Ring: It is a five-membered lactam ring (mainly, 2-pyrrolidinone) that function as the central core of the drug molecule.

- Propyl side chain: It is a straight chain having three- carbons (n-propyl) connected to the fourth carbon of the pyrrolidine ring.

- Butanamide side chain: It contains an acetamide group attached with an ethyl group, linked to the nitrogen atom in thepyrrolidine ring [3].

Stereo chemical complexity: Brivaracetam contains two chiral centers, one at the C2 position of the butamide side chain and other at the C4 position of the pyrrolidine ring.

This results in its four possible stereo isomers:

- (2S, 4R): This is the only stereoisomer which exhibits therapeutic activity of Brivaracetam.

- (2S, 4S), (2R, 4S), and (2R, 4R): These are the other three stereoisomers which not exhibits any therapeutic activity. So, these are considered as stereoisomeric impurities [6, 10]. To separate these isomers is a very challenging in analytical chemistry. Specialized methods, such as chiral or highly selective techniques like RP-HPLC, are needed to ensure that the drug product contains only its active form (2S, 4R) and not its stereoisomeric impurities (2S, 4S), (2R, 4S) and (2R, 4R) [10, 1].

Physicochemical properties: The following properties influence the brivaracetam's behavior in liquid chromatography and its stability in various formulations:

Table 1. Physicochemical properties of Brivaracetam

Property	Description/ Value
Molecular Weight	212.29 g/mol
Physical appearance	White to off-white crystalline powder
Solubility	Highly soluble in water, methanol, and acetonitrile
pKa	Weakly basic/ neutral; lacks strongly ionizable groups
Log P	~1.04 (indicating moderate lipophilicity)
Melting Point	Approximately 72ºC-78ºC

6. MATERIALS AND METHODS:

The development and testing of a method to check the stability of Brivaracetam by using HPLC it needs step by step approach. This involves use of high quality chemicals, appropriate lab tools and following ICH guidelines [12, 11].

Reagents and chemicals:

The quality of reagents and chemicals used in the technique is very important for getting accurate results.

- Reference standard: A Brivaracetam standard with more than 99% purity is generally obtained from relable chemical suppliers [5, 10].

- Solvents: Solvents of high quality such as Acetonitrile and Methanol are used to decrease background noise and avoids incorrect or fluctuated peaks [1, 14].

- Buffers and reagents: Salts of high grade like Potassium dihydrogen phosphate, Ammonium acetate, and Orthophosphoric acid are used to control the pH of the liquid components of the mobile phase [19].

- Water: In this method, Ultra pure water also called as Milli-Q water with a resistance of 18.2 MΩ.cm, is used for the all solutions which are water based [31].

Instruments and Chromatography setup:

Choosing the appropriate equipment and making small, precise and calculated adjustments to a system to optimize performance, accuracy, or efficiency are key for separating Brivaracetam from its breakdown products [4, 15].

- Chromatography system: Brands such as Agilent, Waters, or Shimadzu with a photodiode array (PDA), UV detector are the HPLC systems use by the analysts.

- Stationary phase: The most common type of column used in HPLC system is C18 (Octadecylsilane) column. It is available in sizes like 250 × 4.6 mm or 150 × 4.6 mm, having a particle size of 3-5 µm. This column helps to keep the pyrrolidine part of Brivaracetam in the right place [15, 10].

- Mobile phase: Techniques are generally developed using isocratic elution for simple tests [17, 19] or gradient elution for checking impurities.

- Operating conditions:

a. Flow rate: It is kept between 0.8 and 1.2 ml/min [1, 5].

b. Wavelength: Detection is generally done at 210 nm or 215 nm [10, 14].

c. Injection volume: It is fixed at 10-20 µl [19].

d. Column temperature: Kept at room temperature or a little higher amount such as 30-40°C, to improve peak shape [2].

Preparing solutions:

- Standard stock solution: A specific amount of Brivaracetam is dissolved in a mixture of water and an organic solvent to get a concentration of 1 mg/ml [15, 10].

- Sample solution: For tablet samples, the tablets are ground. An amount matching the dose is extracted, is filtered using a 0.45 µm filter, and then it is diluted to the required concentration [19, 14].

7. ELUCIDATION OF FORCED DEGRADATION BEHAVIOR OF BRIVARACETAM:

When the Brivaracetam is exposed to different stress conditions, it gets breaks down in different ways. This happens due to its specific structure and chemical properties. The Brivaracetam molecule has an amide group in its butamide side chain and this plays an important role in its degradation.

Degradation under alkaline conditions:

Degradation under alkaline conditions happens because of base-catalyzed amide hydrolysis. In basic environments, hydroxide ions act as strong nucleophiles and attack the carbonyl carbon in the amide linkage. This results in breakage of the amide bond which further forms acidic and amide-based degradation products. This reaction is very faster in basic conditions than in acidic conditions, which explains why more degradation is seen in alkali stress tests [18].

Acidic conditions:

In acidic conditions, amide hydrolysis occurs when the carbonyl oxygen is protonated which makes the carbonyl carbon more electrophile. Therefore, this pathway is slower than the base-catalyzed pathway, so the degradation of Brivaracetam under acidic conditions is moderate [4].

Oxidative degradation:

In this case, Brivaracetam degrade through N-oxidation reactions when it is exposed to hydrogen peroxide. The nitrogen atoms in the Brivaracetam molecule are responsible for oxidative attack, it cause to the formation of oxidized byproducts, specifically at higher concentrations of peroxide.

Thermal and photolytic degradation:

Brivaracetam is generally stable under heat and light, study shows that there are no thermally unstable or strongly light sensitive parts in its structure. Minor degradation may occurs with long time exposure to heat or light because of slower secondary reactions rather than direct breakdown of the molecule's structure [16].

8. METHOD VALIDATION PARAMETERS:

Method validation is done as per ICH Q2(R1) guidelines to make the developed analytical technique reliable, precise and suitable for its intended use [11, 8].

Linearity and range:

Linearity is tested over a concentration range of 50 to 150% of the target assay concentration. According to ICH Q2(R1), the method should show a clear linear relationship between the amount of the substance being measured and the response from the detector across this range. The general standard for acceptance is a correlation coefficient (R^2) that is least 0.99. Studies on Brivaracetam show R^2 values that are actually above 0.999, means the method has very good linearity [14].

Accuracy:

Accuracy is tested by doing recovery studies at three different concentration levels, such as 80%, 100% and 120% for an assay method. In research studies, on Brivaracetam it shows that the tablet recoveries within this range, showing that the method is accurate [19].

Precision:

Precision checks both repeatability (how consistent results are when run on the same day) and intermediate precision (how consistent results are when run on different days). ICH Q2(R1) suggests that the percentage relative standard deviation (%RSD) for precision tests should generally not be more than 2.0% for an assay method. Validated techniques for Brivaracetam drug meets with this standard, with % RSD values generally below 2.0%, showing the method is precise [15].

Limit of detection and limit of quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) tell us how sensitive the analytical technique is. ICH Q2(R1) says that LOD and LOQ can be determined by looking at the signal to noise ratios, around 3:1 for LOD and 10:1 for LOQ, or by using the standard deviation of the response and the slope of the calibration curve. UPLC techniques for Brivaracetam generally have lower values as compared to traditional HPLC techniques, making them better at detecting small amounts of impurities and breakdown products [5].

Robustness:

Robustness checks how accurate the method is when there are small purposeful changes in the method's conditions. According to ICH Q2(R1), the technique should still work well even if there are minor changes in chromatography conditions. Common robustness tests include changing the flow rate ±0.1 ml/min, the mobile phase composition by ±2%, or the pH by ±0.2 units. There is no major effect on system suitability or assay results, which proving that the method is robust [1].

9. ESTIMATION OF BRIVARACETAM IN PHARMACEUTICAL DOSAGE FORM

To find the average weight of 20 tablets, crush them by using a mortar and pestle. Take a portion of the powder that weighs 25 mg and transfer it into a 25 ml volumetric flask. Sonicate the mixture for 15 minutes, then fill the flask to the 25 ml mark with the mobile phase. Then, take 10 ml of this solution and dilute it to 100 ml. Record the results. The data are presented in Table 2. The amount of drug in Briviact tablet was found to be 49.867 (± 0.468) mg per tablet for Brivaracetam, and the purity was found to be 99.825%. Different chromatographic conditions were used to develop a precise, linear, specific, and stable RP-HPLC method for analyzing Brivaracetam. Isocratic elution is simple which requiring only one pump and providing a flat baseline for consistent results. Because of this, it was selected over gradient elution for this study [20].

Table 2. Estimation of Brivaracetam in pharmaceutical dosage form

Brand name	Labelled amount (mg)	Mean	Assay%
Briviact	50 mg	50.10	100.34

10. FUTURE TRENDS AND DISCUSSIONS:

The way we test Brivaracetam is becoming more eco-friendly. In 2025, new multi-task HPLC methods were developed which uses less amount of solvent and fewer harmful chemicals, while keeping the same level of accuracy in testing [2]. Study shows that Brivaracetam is stable in neutral conditions but breaks down a lot in very acidic or very alkaline conditions [12]. Transferring it from HPLC to UPLC has made these tests better at showing how stable Brivaracetam is because it can separate more breakdown products in less time [5]. Also, by using Mass spectroscopy (MS) it has switched the focus from just finding breakdown products to understanding how the Brivaracetam drug molecule changes chemically [20].

11. CONCLUSION:

This review studies ten years of research that shows how important it is to create and check stability-indicating methods, not just because it is required, but because it is essential for making sure drugs are safe and work well. Scientists followed strict rules from ICH Q1A(R2) ans Q2(R1) to build strong testing methods that can find Brivaracetam accurately even in mixtures of other substances [7, 8]. One great discovery from this review is how the Brivaracetam breaks down. The data shows Brivaracetam can expose to heat

and light but it is highly sensitive to strong bases and oxidation [18, 4]. When Brivaracetam is exposed to basic conditions it get turns into a carboxylic acid, which means it needs to be stored and prepared in a controlled pH environment. By moving from old RP-HPLC methods to newer, faster techniques like UPLC and LC-MS/MS has made it easier and more accurate to spot small impurities [5, 4].

Another research study shows the different forms of Brivaracetam called as enantiomers. Currently, methods can clearly tell the active (2S, 4R) form apart from the other three ones, which helps the drug to keep its strong bond with SV2A, a key part of its effectiveness [6, 3]. In 2025, new green chemistry methods showed that the high-quality testing can be done without hurting the environment [2]. In short, the HPLC and UPLC methods are specific, accurate, and reliable enough to support drug approvals worldwide and regular quality checks. Future work should connect industrial testing with clinical analysis, maybe by developing automated systems for real-time stability checks and by improving green methods to make Brivaracetam production more ecofriendly.

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