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Systematic Development of UV Spectrophotometric Method for Brivaracetam Analysis

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ABSRACT:
The disorder of epilepsy is characterized by recurrent, uncontrollable convulsions as well as other co-morbidities. An episode of convulsions results from abnormal brain activity that causes symptoms and indications to appear for a brief period. In the current study a new, straightforward, accurate, simple, precise, and relatively inexpensive ultraviolet (UV) spectrophotometric technique with improved detection range was developed. After the literature survey, we found that only one method was reported for the estimation of Brivaracetam, which aimed us to develop other different methods by using a UV spectrophotometer. The maximum absorbance of brivaracetam was found at λ_{max} 217nm. Brivaracetam was shown to be linear in the UV spectroscopic approach over the concentration range of 10–50 g/ml, with a correlation coefficient of 0.998721. The procedure has been statistically examined and the findings were within the appropriate limits. A linear regression equation was obtained using the least squares method with the following values: y = 0.053x + 0.079 with an R^2 value of 0.997 within the range. The procedure was shown to be accurate, precise, reproducible, and simple to use, and to be economical as well as less time-consuming with respect to the results. According to ICH guidelines (ICH Q2 (R1)), the analytical method was developed and validated using all validation parameters to meet criteria including accuracy, precision, linearity, reproducibility, interday precision, intraday precision, robustness, the limit of detection and limit of quantitation was found to be within acceptable limits and the analysis results were validated statistically.

KEY WORDS: Brivaracetam, Ultraviolet spectrophotometry, validation, Anti-epileptic drug, development

I. INTRODUCTION

Epilepsy is a disorder characterized by recurrent and uncontrolled convulsions, as well as other co-morbidities. A convulsion is a brief episode of symptoms or indications caused by abnormal brain activity. [1] Brivaracetam, a third-generation anti-seizure racetam analogue with high selectivity when acting as a ligand for the synaptic vesicle protein 2A (SV2A) its affinity for SV2A is more than other drugs, [2] BRV is a more recent antiepileptic drug (AED) for the supplemental medical treatment of neurological impairments that result in generalization of seizures. [3] The two different types of epileptic fits are generalized seizures, which affect the whole brain simultaneously and localized seizures, which affect just one area of the brain. [4] It is an even more potent medicine and in certain instances is much more effective than other seizure medications. It offers a broad spectrum of anti-epileptic effects for both focal and generalized epilepsy. [6] It is a considerably more effective as compared to existing anti-convulsant drugs. For both focal and generalized epilepsy a wide range of anti-epileptic actions are available.[5] In addition it have a faster time of entry into the nervous system and also considered as a precursor to levetiracetam, along with 20 percent stronger affinity at the SV2A modulator. Brivaracetam also has the advantage of being able to be delivered intravenously for a more rapid brain immersion than other third-generation AED's. [6] In the brain, Brivaracetam do have a greater transparency and is also more lipophillic in nature. [7] According to *Gillard et al*., Brivaracetam

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(BRV) is an acute and attractive ligand that has been approved as a secondary therapy for recurrent seizures in elderly people with epilepsy. Phase-III assessments on individuals with recurrent seizures have also shown the positive effects of brivaracetam with 50 mg to 200 mg each day as an adjuvant treatment in case of geriatrics and pediatrics starting at the age of 4. [8] In randomized controlled trials (RCTs), brivaracetam reduces the frequency of seizures in patients with resistant to drugs temporal lobe epilepsy when combined with existing anti-seizure drugs (ASMs). [9] Brivaracetam is also known by its chemical name, (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide. The lactam ring/loop contains a propyl chain, which boost its responsiveness to the interaction domain on SV2A, making it different from leviracetam. Its molecular weight is 212.293 g/mol, and its chemical make-up is C11-H20-N2-O2. [10] The amount of BRV in the cerebral spinal fluid (CSF) reaches its maximum level within ten minutes after being administered intravenously. [11] Racetam drugs, such as brivaracetam inhibit calcium-dependent exocytosis of synaptic vesicles containing excitatory neurotransmitters thereby reducing the chances of partial seizures. [12] It affects 65 million people worldwide and is one of the most prevalent chronic neurological diseases. In spite of the fact that antiepileptic drugs (AEDs) are effective in treating many sick people, at least one third of them develop pharmacoresistant epilepsy. [13]

II. MATERIAL AND METHODOLOGY

INSTRUMENTS USED:

Sr.No	Instrument	Company	Model
1.	UV Spectrophotometer	Labindia	3000^{+}
2.	Weighing balance	Wensar	PGB200
3.	Sonicator	Rolex India	PS-10A

CHEMICAL USED:

Brivaracetam was purchased from Ravenbhel Healthcare Private Limited; Methanol was purchased from Sigma Aldrich, Missouri and United States.

SELECTION OF SOLVENT:

100 mg of Brivaracetam was accurately weighed and transferred to 10 ml of volumetric flask. Various solvents were used as solvent & were marked up to the volume of the volumetric flask that was used to check the solubility of the drug. The results are shown in **table 1.1.**

Table: 1 Solubility of Brivaracetam drug

Sr. No.	Solvent	Brivaracetam
1.	Methanol	Very Soluble
2.	Ethanol	Very Soluble
3.	Acetonitrile	Freely Soluble
4.	Acetone	Freely Soluble
5.	Distilled water	Soluble
6.	Toluene	Soluble
7.	n-hexane	Slightly Soluble

2.2.2 SELECTION OF ANALYTICAL WAVELENGTHS:

Appropriately dilutions was prepared for the given drug from the standard stock solution and scanned in the spectrum mode in the UV spectrophotometer from 200 to 600 nm. Brivaracetam showed maximum absorbance at 217 nm respectively.

2.3 PREPARATION OF STOCK SOLUTION:

2.3.1 BRIVARACETAM STANDARD STOCK SOLUTION-

Standard Brivaracetam 100 mg was accurately weighed and was transferred to a 100 mL of volumetric flask and was dissolved in suitable solvent i.e. MeOH. The flask was then shaken and volume was made up to the mark with the solvent (Stock-I Solution).

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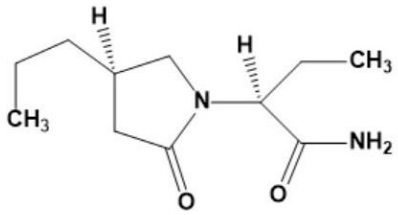
Then 10 mL was pipetted out from the above solution i.e., from the Stock-I solution and transferred to 100 mL volumetric flask with methanol as solvent (Stock –II Solution). The concentration of prepared stock solution was 100 µg/mL



Brivaracetam

Fig. 1. Chemical structure of Brivaracetam.

2.3.2 SELECTION OF ANALYTICAL CONCENTRATION RANGES-

From the standard stock solution of Brivaracetam (100 µg/mL) 1,2,3,4,5 mL was pipetted out and transferred to separate 10 mL of volumetric flask and volume was made up to 10 mL with the help of the suitable solvent that is methanol. These concentrations were of 10, 20, 30, 40, 50 µg/mL respectively. Absorbance for the solution was measured at 217 nm for Brivaracetam drug.

A calibration curve of absorbance versus concentration was plotted. Brivaracetam drug follows the Lamberts Beer law in the range of 10 to 50 µg/mL.

III. METHOD VALIDATION PARAMETER

3.1 LINEARITY CURVE FOR THE BRIVARACETAM (2-10 µg/ml)

From the standard stock solution of brivaracetam (100 µg/mL) the volume of 1, 2,3,4,5 mL was pipetted out and transferred to different 10 mL of volumetric flask and the volume was made up to 10 mL with the help of methanol as a solvent up to the mark. These concentrations were of 10, 20, 30, 40 and 50 µg/mL respectively. Absorbance of each solution against given solvent as blank was measured at 217 nm. The graph of absorbance versus concentration was plotted. The regression equation and correlation was determined in every case. Results were shown in **Table. 2**

3.2 ACCURACY:

Accuracy is also termed as Trueness. By an analytical procedure accuracy indicates the degree of agreement between the accepted value and the value found with respect to both conventional true value and accepted reference values. Accuracy may often express in terms of percent recovery of assay of known amount of analyte added. Recovery studies were carried out by addition of standard drug to the sample at three different levels of spiking i.e. 80%, 100% and 120% of the actual amount taking into consideration percentage purity of added bulk drug samples.

3.2.1 ACCURACY FOR BRIVARACETAM DRUG

For brivaracetam three level of spiking i.e. 80%, 100%, 120% was done of each prepared by adding 0.8mL, 1 mL, 1.2 mL in 10 mL of volumetric flask respectively. The three replicates of each dilution were prepared for brivaracetam for respective three days. Absorbance of each of the concentration was taken accurately and accuracy was calculated as % drug recovery was calculated according to ICH guidelines for the taken drug. The results were show in the **Table.3**

3.3 PRECISION:

The precision of an analytical method is the degree of closeness of agreement between a series of measurements obtained from the multiple sampling of the same sample. Precision include repeatability, interday precision and intraday precision and reproducibility.

3.3.1 INTERDAY & INTRADAY PRECISION:

Interday & intraday precision of concentration 10, 20, 30, 40, 50 µg/mL were prepared in three replicates from the stock solution and absorbance was taken. The result was shown in **Table.4** for intraday precision. For interday precision the result was shown in **Table. 4.1**

The absorbance for intraday was measured in 2 hours of interval for each set.

3.3.2 REPEATABILITY:

In case of repeatability of brivaracetam minimum of 6 determinants were prepared of 40 µg/mL concentration and the absorbance was taken at 217 nm and the result was shown in **Table. 4.2**

3.3.3 REPRODUCIBILITY:

For reproducibility three determinants of 30 µg/mL were prepared and inter laboratory trials were taken at 217 nm and results were shown in the **Table. 4.3**

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The standard deviation and relative standard deviation were reported for each type of precision.

3.4 LIMIT OF DETECTION:

Limit of detection (LOD) is an individual analytical method which can be disclosed easily but not fundamentally quantitated as an accurate worth and is the lowest amount of analyte in a specimen. LOD was calculated by the standard deviation of the response and the slope

The limit of detection (LOD) may be expressed as:

$$LOD = \frac{3.3 \times \sigma}{S}$$

Where;
σ = Standard deviation of the response
S = Slope of the calibration curve
The slope and the standard deviation were calculated from the linearity curve obtained from concentration ranges of 10 to 50 µg/mL for brivaracetam drug. The result was shown in the **Table. 5** for brivaracetam.

3.5 LIMIT OF QUANTITATION (LOQ):

The quantitation limit or LOQ refers to the amount or concentration of a substance that can be determined using a certain analytical procedure within the established degree of accuracy, precision, and uncertainty of a given analytical procedure, regardless of the amount or concentration it is possible to determine. LOQ was calculated by the standard deviation of the response and the slope. The data was obtained from linearity curve and the LOQ was calculated.
The limit of quantitation (LOQ) may be expressed as:

$$LOQ = \frac{10 \times \sigma}{S}$$

Where;
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope and the standard deviation were calculated from the linearity curve obtained from concentration ranges of 10-50 µg/mL for brivaracetam drug. The result was shown in the **Table. 6** for brivaracetam.

3.6 ROBUSTNESS:

Robustness is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameter. The robustness of the method was checked by changing the wavelength. There was change of ± 2 nm wavelength. Three concentration of 30, 40, 50 µg/mL were prepared with their 3 replicates and absorbance was measured. The result was shown in **Table. 7** for brivaracetam.

IV. RESULTS

The absorption maxima of drug was found to be at 217nm as shown in **Fig. 1**. This wavelength was further used for the validation of analytical methods.

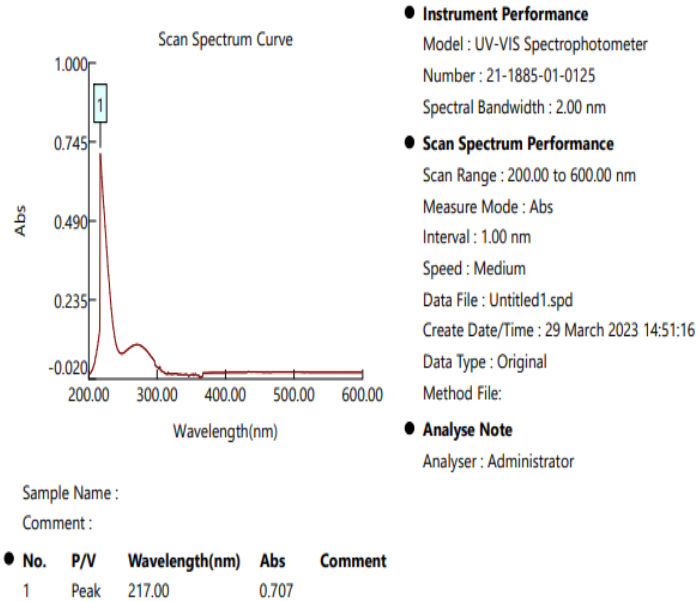

Fig. 1: UV Spectrum of Brivaracetam.

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Table. 2 Result of linearity in UV spectrophotometer

Concentration (ug/ ml)	Absorbance (nm)
10	0.177
20	0.300
30	0.412
40	0.510
50	0.608

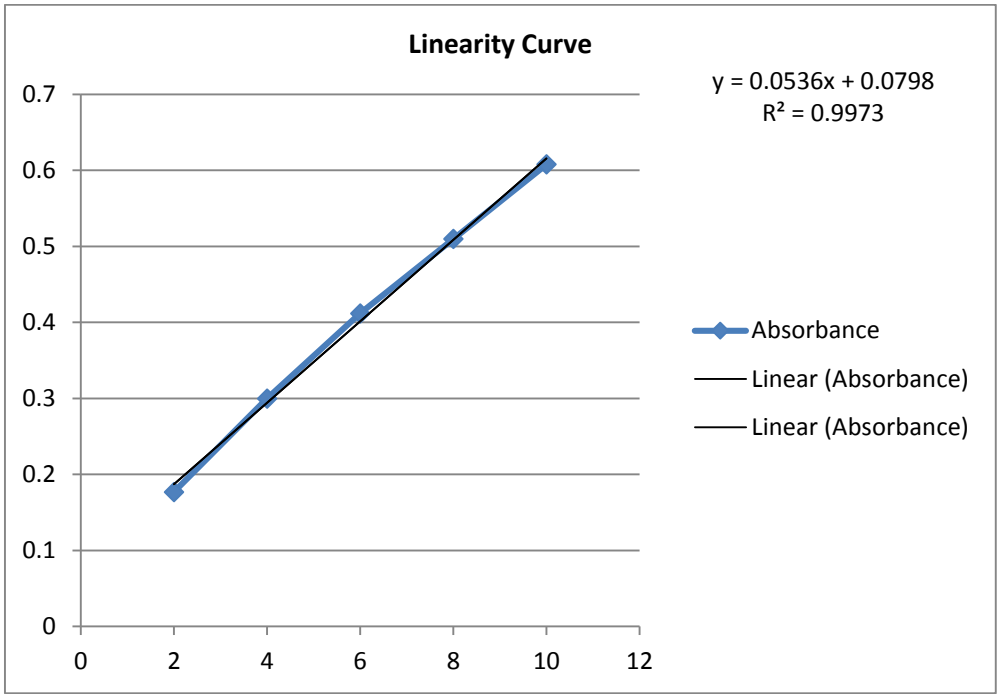

Table.3 Result of Accuracy in UV spectrophotometer

Spiked level	Concentration (µg/ ml)	Absorbance (nm)
80%	10	0.138
	10	0.136
	10	0.140
100%	10	0.133
	10	0.135
	10	0.132
120%	10	0.155
	10	0.152
	10	0.151

Mean(80%)	0.138	**S.D**	0.002	**%RSD**	1.449275
Mean(100%)	0.133333333	**S.D**	0.001528	**%RSD**	1.145644

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Mean(120%)	0.152666667	**S.D**	0.002082	**%RSD**	1.363537

Table. 4 Result of Intraday precision in UV spectrophotometer

Concentration(µg/ml)	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
Absorbance(nm)	0.166	0.31	0.463	0.592	0.730
	0.168	0.309	0.467	0.586	0.727
	0.164	0.305	0.462	0.593	0.725
Mean	0.166	0.308	0.464	0.5903333	0.7273333
Std. dev.	0.002	0.0026458	0.0026458	0.0037859	0.0025166
%RSD	1.2048193	0.8590102	0.570205	0.6413222	0.3460052

Table. 4.1 Result of Interday precision in UV spectrophotometer

Sr.No.	**Conc. (µg/ ml)**	**Absorbance 1 (nm)**	**Absorbance 2 (nm)**	**Absorbance 3 (nm)**	**Mean**	**Standard deviation**	**%RSD**
1.	10µg/ml	0.166	0.168	0.166	0.166666667	0.001154701	0.692820323
2.	20µg/ml	0.310	0.309	0.310	0.309666667	0.00057735	0.186442498
3.	30µg/ml	0.463	0.461	0.462	0.462	0.001	0.216450216
4.	40µg/ml	0.592	0.590	0.593	0.591666667	0.001527525	0.258173279

Table. 4.2 Result of repeatability in UV spectrophotometer

Sr.No.	**Concentration (µg/ ml)**	**Absorbance1 (nm)**	**Absorbance2 (nm)**	**Absorbance3 (nm)**	**Mean**	**Std dev**	**%RSD**
1.	10	0.166	0.168	0.166	0.166667	0.001155	0.69282
2.	20	0.310	0.309	0.310	0.309667	0.000577	0.186442
3.	30	0.463	0.461	0.462	0.462	0.001	0.21645
4.	40	0.592	0.590	0.593	0.591667	0.001528	0.258173
5.	50	0.730	0.727	0.725	0.727333	0.002517	0.346005

Table. 4.3 Result of reproducibility in UV spectrophotometer

PARAMETERS	**LAB 1**	**LAB 2**
ABSORBANCE (nm)	0.066	0.068
	0.065	0.067
	0.066	0.068
MEAN	0.065666667	0.067666667
S.D	0.00057735	0.00057735
%RSD	0.879213608	0.853226999

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Table. 5 Result of limit of detection (LOD) in UV spectrophotometer

Concentration (µg/ ml)	Absorbance (nm)	ȳ	Residual value R	R2
10	0.091	0.082	0.009	0.000081
20	0.146	0.142	0.004	0.000016
30	0.212	0.202	0.01	0.0001
40	0.277	0.262	0.015	0.000225
50	0.345	0.322	0.023	0.000529
				0.00019

The % RSD was found to be 0.000475 and the value of LOD was found to be 0.026125.

Table. 6 Result of limit of quantitation (LOQ) in UV spectrophotometer

Concentration (µg/ ml)	Absorbance (nm)	ȳ	Residual value R	R2
10	0.091	0.082	0.009	0.000081
20	0.146	0.142	0.004	0.000016
30	0.212	0.202	0.01	0.0001
40	0.277	0.262	0.015	0.000225
50	0.345	0.322	0.023	0.000529

The % RSD was found to be 0.0000475 and the value of LOQ was found to be 0.07916.

Table. 7 Result of robustness in UV spectrophotometer

Conc. (µg/ ml)	Replicates	Absorbance (nm)
30µg/mL	2	0.212
	2	0.21
	2	0.214
40µg/mL	2	0.277
	2	0.277
	2	0.279
50µg/mL	2	0.356
	2	0.355
	2	0.357

This method was checked by changing different wavelength. In this we have taken three concentrations that is 30, 40, 50 µg/mL was prepared along with their three replicates and absorbance was measured.

V. CONCLUSION

For determining and estimating the specific method of brivaracetam drug, the Ultraviolet spectrophotometric method has been developed and validated. A validated method was developed according to ICH guidelines and had to meet criteria including accuracy, linearity, precision, interday precision, intraday precision, reproducibility, repeatability, the limit of detection, the limit of

quantitation, and robustness. A linear calibration curve was obtained over a concentration range of 10-50 µg/ml. A relative standard deviation (RSD) of less than 1% was found in the case of linearity, and precision and also in the case of accuracy relative standard deviation was found less than 2%. A fast, sensitive, accurate, and efficient method is proposed that can be used to analyze samples in laboratories.

In UV visible spectrophotometry there is no method developed for quantitative determination of brivaracetam in samples and its preparations by using different parameters. But in this research work, we are developing and validating a UV visible spectrophotometric technique for brivaracetam in order to meet our goals of rapid, affordable, and accurate quantification of brivaracetam.

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