IOSR Journal Of Pharmacywww.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 7, Issue 12 Version. 1 (December 2017), PP. 44-48

Chromogenic Spectrophotometric Estimation of Brivaracetam in Bulk drug & its Formulation with Folin Ciocalteu reagent

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ABSTRACT:
Simple, precise, economic & less time consuming visible spectroscopic method for Brivaracetam was developed by using Folin Ciocalteu Reagent (FCR) in the presence of base. The absorption maximum of the chromogen was found to be 475 nm. The developed method was obeying Beer Lambert's law in the range of 2-20 µg/ml concentration. The method has also been statistically evaluated & the results were within the limits. Molar absorptivity, Sand ell's sensitivity a& Correlation –co-efficient were found to be 1.528 x 10^3, 0.109 and 0.998 respectively & the method is free from the interferences of other additives present in the formulation.
Key Words: Brivaracetam, Folin Ciocalteu Reagent (FCR), Quantification, Chromogenic

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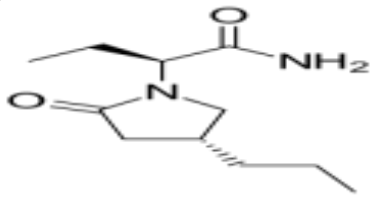
Date of Submission: 06-12-2017 Date of acceptance: 21-12-2017

I. INTRODUCTION

Brivaracetam [(2S)-2-[(4R)-2-oxo-4-propyl-tetrahydro-1H-pyrrol-1-yl]butanamide] used in the treatment of the partial-onset seizures[1, 2]. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicle glycoprotein [3]. Brivaracetam (Briviact), a chemical analog of levetiracetam [4, 5]. Chemical Structure of Brivaracetam was shown in the Figure 1.Only few HPLC/UPLC-MS/MS methods for quantification of Brivaracetam were reported in the literature & these methods were applied in the determination of Brivaracetam & are mainly useful for therapeutic drug monitoring [6, 7]. No visible spectrophotometric method for quantitative determination of Brivaracetam in bulk drug samples and its formulations was reported in the literature. The aim of the current work is to develop and validate rapid, economical and sensitive visible spectrophotometric method for quantitative determination of Brivaracetam in bulk drug samples and its formulations.

Fig.1: Structure of Brivaracetam



II. EXPERIMENTAL

2.1 Instruments

SHIMADZU-1700 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements.
Digisun model DI-707 pH meter was used for all the pH measurements.

2.2 Chemicals and Reagents: All the chemicals used were of analytical grade.
Folin Ciocalteu (33.3 % v/v): It is prepared by dissolving 33.3 mL of FC reagent in 100 ml of distilled water.
Sodium Hydroxide (0.1N): It is prepared by dissolving 0.4 gms of NaOH in 100 ml of distilled water.

2.3 PROCEDURES:

2.3.1 Preparation of Standard drug solution:

A standard drug solution of Brivaracetam was prepared by dissolving 100 mg of drug in 100 ml of water in a standard volumetric flask to obtain a stock solution of 1 mg/mL

2.3.2 Bulk Drug Samples Estimation:

Aliquots of standard solutions were taken for preparing 2-20µg/ml solutions. Suitable aliquots were transferred into respective labeled test tubes, to each of it 1ml of 33.3%V/V Folin Ciocalteu and 1ml of 0.1N NaOH were added. These solutions were heated at less than 60^{o}c for 5 minutes. The volume was made up to 10ml with distilled water. The absorbance of the colored species was measured at 475 nm against reagent blank. The colored species was stable for more than 24hrs. The amount of Brivaracetam present in the bulk sample was computed from its calibration curve.

2.3.3 Estimation of Brivaracetam in Formulation:

Sample solution was prepared by dissolving 100 mg equivalent of Brivaracetam tablet powder in 100ml of water. The working standard solution of 1000 µg/ml is prepared. The prepared solution was kept in sonicator for sonication for 10 mins, same procedure applied as above to prepare, measure the absorbance at 475nm. Various formulations available in the market are described in Table 2. The amount of Brivaracetam present in the bulk sample was computed from its calibration curve with extrapolation of absorbance value.

III. RESULTS AND DISCUSSION

3.1 Optimization of parameters for Method:

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen. Wave length maximum of chromogen is described in the Fig 2. Brivaracetam has amino group in the molecular structure making it possible to undergo oxidative coupling of the drug with FCR in the presence of base. The effect of temperature of the reaction, quantity, concentration and order of addition of various reagents were studied, optimized after several trials with respect to maximum sensitivity, color stability, adherence to Beer's law and other optimum conditions are incorporated in the procedure. Optical parameters of the method were described in Table 1.

3.2. Optical Characteristics:

3.2.1 Linearity

Linearity was demonstrated in the concentration range of 2-20 µg/ml by using the method of least squares. Regression analysis was performed to evaluate the slope (m), intercept (b) , correlation coefficient (r^2), Molar absorptivity, Beer's law limits, absorption maxima and Sandell's sensitivity were presented in Table.1.The graph showed negligible Y-intercept. Calibration curve was shown in Fig.3.

Table 1: Optical characteristics

01	λ_{max} (nm)	475
02	Beer's law range (µg/ml)	2-20
03	Molar extinction coefficient(L.mole^{-1} cm^{-1})	1.528×10^3
04	Sandell's sensitivity (µg/cm^2/0.001)	0.109
05	Regression equation (y = mx + c) * Slope (m) Intercept (c)	 0.026 0.131
06	Correlation coefficient (r)	0.998
07	Precision (%Relative Standard Deviation)	0.269
08	Standard Error of Mean	0.37771

Table 2: Commercially Available Formulations of Brivaracetam .

Generic Name	Proprietary Name	Dosage Form	Content
Brivaracetam	Breviact , UCB Pharma Limited	Tablets	10mg,25mg
	Breviact , UCB Pharma Limited	Tablets	50 mg
	Breviact , UCB Pharma Limited	Tablets	75mg and 100mg

Fig 2. Absorption Spectrum of Brivaracetam with FC

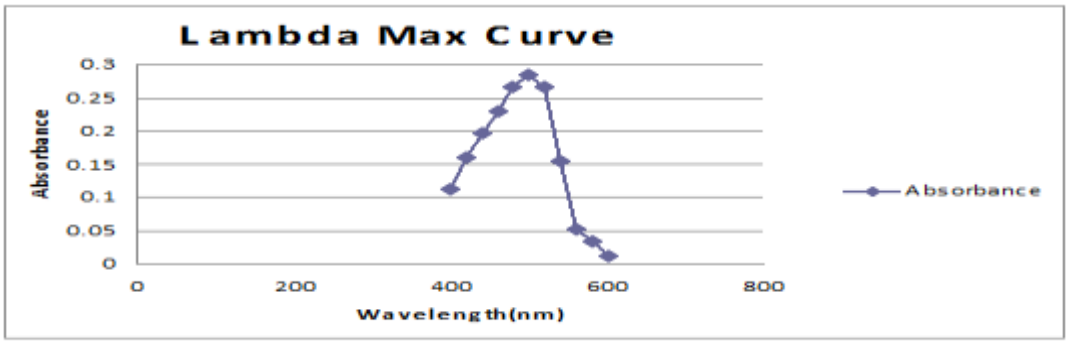
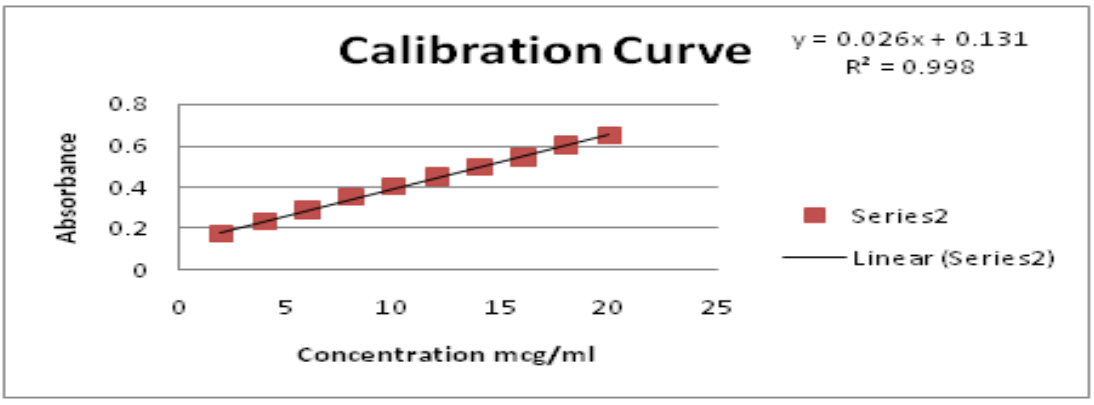


Fig 3: Calibration Curve of Brivaracetam



3.2.2 Accuracy and Recovery

Commercially available tablets of Brivaracetam (Table 3) were analyzed by the proposed method and as additional check on the accuracy of the method, recovery experiments were also conducted by spiking known amounts of pure drug in preanalysed formulation and the recovery was calculated in each of the case using the regression line equation developed under the Linearity experiment. Assay results of the proposed method was compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The means of the proposed method are not significantly different from that of reference method ($P > 0.05$). The assay & accuracy results were presented in Table.3. The interference studies indicated the common additives and excipients present in formulations did not interfere with the proposed method.

Table 3: Evaluation of dosage forms (n=6)

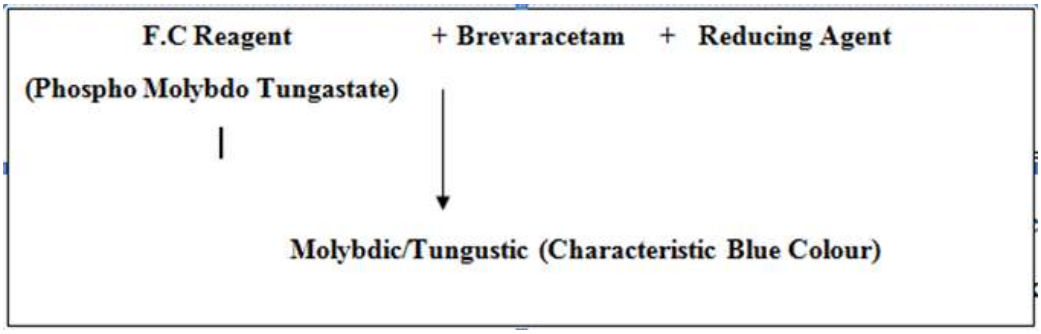
Sample[a]	Labelled Amount (mg)	Amount obtained (mg)		Percentage Recovery
		Proposed method	Reference method [6]	Proposed Method
B_1	50	49.05±0.47	48.07±0.72	98.10±0.12
B_2	50	49.80±0.12	49.62±0.20	99.60±0.17

a - B_1 and B_2 are the tablets from different batches
b – Mean ± SD of 6 determinations.
c – 40 mg of pure drug was added and recovered.
For the sample One-way ANOVA with post-test followed by Dunnett multiple comparison tests were performed. The results showed that P > 0.05 & means of the proposed method are not significantly different from that of reference method.

IV. CHEMISTRY OF THE COLORED SPECIES FORMED

As Brivaracetam contains an amino functional group, it can be analysed using Folin Ciocalteu Reagent effectively. The colour formation by FC Reagent may be explained in the following manner based on the analogy, proposed Scheme of complex formation was described in the Fig 4. FC reagent contains $3H_20.P_2O_5.13WO_3.5MoO_3.10H_2O$ & $3H_20.P_2O_5.13WO_3.4MoO_3.10H_2O$. Amines probably effects a reduction of 1, 2 or 3 oxygen atoms from Tungstate & molybdate in FCR thereby producing characteristic blue colour.

Fig 4 Proposed Scheme of complex Formation



V. CONCLUSION

The proposed Chromogenic spectrophotometric method enables quantitative determination of Brivaracetam in bulk drug samples & its formulation. Efficient quantification at the respective absorption maxima enabled determination with no interference from the other excipients present in the formulation. The calibration curve was linear over a concentration range from 2-20 µg/ml. The relative standard deviation (R.S.D.) was less than 1% and average recovery was in the range of 98-102%. The proposed method is fast, sensitive, precise, accurate & efficient and can be used for analysis in quality control laboratories.

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Imam Pasha.S "Chromogenic Spectrophotometric Estimation Of Brevaracetam In Bulk & Its Formulation With Folin Ciocolteus Reagent." IOSR Journal of Pharmacy (IOSRPHR), vol. 7, no. 12, 2017, pp. 44-48