Stability Indicating Thin-Layer Chromatographic Determination of Brivaracetam as Bulk Drug: Application to Forced Degradation Study

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Abstract: A new, accurate, selective, simple and precise HPTLC method for estimation of brivaracetam in bulk drug as well as tablet dosage form was developed and validated. The drug was well separated using mobile phase of ammonium acetate: methanol:n-propanol (8:1.6:1.6 v/v/v) with densitometric quantification of brivaracetam at 242nm. The TLC parameters were standardized and the Rf of brivaracetam was determined to be 0.40. The values of Linearity (200-1200ng/spot), Method precision (intra-day RSD 0.5-0.8% and inter-day RSD 0.25-0.46%), Accuracy (% recovery 97.53%-102.84%) and specificity were determined according to ICH guidelines. Brivaracetam was exposed to various stress condition to study degradation profile. Degradation was seen in acidic, basic and oxidative condition. Brivaracetam was found to be stable in photolytic condition. The experiment gives us satisfactory result for method validation and development indicates the successful validation of HPTLC method for quantitative determination of brivaracetam. The HPTLC method is simple, rapid, economic and more suitable for routine analysis of brivaracetam in bulk and tablet dosage forms.

Keywords: Brivaracetam, HPTLC, Densitometric estimation, Method development, Validation, Stability indicating method.

Introduction

Brivaracetam is a third-generation antiepileptic racetam derivative and a 4-n-propyl analogue of levetiracetam [Fig.1]. It was granted Food and Drug Administration (FDA) approval as an add-on therapy in February 2016. Brivaracetam is the drug which binds to the synaptic vesicle glycoprotein 2A (SV2A).1-3 In phase II clinical trials the adult patients shows promising effect on refractory partial seizures. Results of stage III trials have been recorded; it is proved that brivaracetam is 10 times more potent for the prevention of certain types of seizure in mouse models than levetiracetam, of which it is an analogue. It shows activity as a novel high-affinity synaptic vesicle protein 2A (SV2A) ligand, displays inhibitory activity at neuronal voltage-dependent sodium channels, data which are collected from animal models suggested potent and broad-spectrum antiepileptic activities.4-6

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There is no known information in the literature about development and validation of brivaracetam by HPTLC. Literature survey reveals data about brivaracetam viz. pharmacokinetics and metabolism of 14C-brivaracetam, metabolism studies of brivaracetam and gemfibrozil, clinical trials of adjunctive brivaracetam for refractory partialonset seizures, identification of drug metabolites in human plasma or serum integrating metabolite prediction by LC–HRMS methods, HPLC Studies on degradation behavior of brivaracetam, a meta-analysis of placebo-controlled studies and development of validated stability indicating method by HPLC and UPLC of brivaracetam.

Currently, HPTLC is used for the identification and quantification of pharmaceuticals. HPTLC offers several advantages include abbreviated analysis time, parallel processing of samples, minimal maintenance cost, modest consumption of mobile phase, etc. Because of the high sensitivity and its utility HPTLC test results was also included in pharmacopoeia; thus HPTLC getting wide acceptance.

The main aim was to develop simple, precise and robust analytical procedure with degradation studies for estimation of brivaracetam and formulation by HPTLC. The proposed method was validated as per ICH guidelines.

![Figure 1: Structure of brivaracetam](image)

**Materials and Methods**

**Reagents and chemicals**

Brivaracetam (99.85%) of pharmaceutical grade was procured from Manus Aktteevva Biopharma LLP, Ahmedabad, India. Silica gel 60F \textsubscript{254} TLC plates (10×10 cm, layer thickness 0.2 mm, Merck, Germany) were used as stationary phase. The pharmaceutical dosage form (Brivact, tablet 25mg, Sanofi India) was procured from local market. All chemicals and reagents were of analytical grade were purchased from Merck (Pvt) Ltd.

**HPTLC Instrumentation and optimization of chromatographic condition**

Pre-coated aluminum plates of silica gel 60F\textsubscript{254} plates (10 cm×10cm) with 250μm thickness (E. Merk, Darmstadt, Germany), supplied by Anchrom technologist, Mumbai were used as stationary phase. The standard solutions were spotted in the form of bands and were applied with the help of Camag 100μl sample (Hamilton, Bonaduz, Switzerland) syringe, on the silica gel plates, using a Camag Linomat V (Switzerland) sample applicator. Prewashing of plates was done with methanol and activated at 110°C for 5min in oven prior to chromatography. Bands were applied with constant application rate of 0.1μl/s with the space of 6mm. The dimension of slit was kept at (5mm×0.45mm) and the scanning speed was 10mm/s. Baseline correction was done and each track was scanned three times.

**Optimization of mobile phase**

Various solvent systems were tried like mixture of a) Methanol: acetonitrile(7:3 v/v) b) Acetonitrile: methanol: toluene (2:2:1v/v/v) c) Toluene: ethyl acetate: methanol (3:1:1 v/v/v) d) Toluene: methanol: ammonia (1:3:0.5 v/v/v) e) Butanol: water (4:1 v/v) f) Ammonium sulphate 0.5% : methanol (8:2 v/v) to separate and resolve spot of brivaracetam from its impurities and other excipients of formulation. The mixture of ammonium acetate: methanol (8:1.6 v/v) could resolve brivaracetam but R\textsubscript{f} value was found in range of (6 to...
Hence to improve Rf value n-propanol 1.6 ml was added in the mixture. The mixture of ammonium acetate: methanol: n-propanol showed satisfactory separation and gives well resolved peak. Rf value of drug was found to be 0.40 ±0.03.

Development of plates was carried out as linear ascending direction in a (20cm×10cm) twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized time period required for chamber saturation was 20 min at room temperature (25°C±2) and relative humidity of 60%±5.

**Forced Degradation Studies**

**Acid and Base Induced Degradation**

Acid and base hydrolysis studies were carried out by exposing drug solution to 0.01N HCl and 0.01N NaOH solution refluxed at 50°C for 15 mins. These solutions was applied on silica plate and developed in the mobile phase. Changes were observed on the densitogram.

**Hydrogen Peroxide-Induced Degradation**

The sample was treated with 3% hydrogen peroxide solution for 2 hours at room. This solution was applied on silica plate and developed in the mobile phase. Changes were observed on the densitogram.

**Photochemical Degradation**

Stock solution (1000 µg/ml) was exposed to direct sunlight for 48 hrs. This solution was applied on silica plate and run the mobile phase. Changes were observed on the densitogram. The photochemical study was also carried out in photostability chamber for 48 hour.

**Experimental**

**Preparation of standard stock solutions**

For the preparation of stock solution accurately weighed 10mg of brivaracetam was transferred to a 10ml volumetric flask and was diluted with methanol (1000µg/ml). From this stock solution 0.1ml was pipetted out and was diluted with 10ml methanol (10µg/ml).

**Prewashing of plates**

The pre-coated silica gel 60F254 plates (10 cm ×10cm) from E. Merck were pre-washed with methanol, dried and activated for 30 min at 110°C.

**Sample application**

The standard and formulation samples of brivaracetam were spotted in the form of bands of length 6mm on pre-coated silica gel TLC plates. With distance of 10mm from the bottom and left margin and 10mm distance between two bands. Samples were applied with the help of continuous drying stream of nitrogen gas at constant application rate of 150nl/s.

**Method Validation**

As per ICH guidelines Q2 9(R1) the developed HPTLC method was validated for various parameters such as linearity, accuracy, precision, limit of detection, limit of quantification, repeatability, specificity and robustness.

**Linearity and calibration curve**

In linearity the method was evaluated with the help of six concentration levels of calibration curves. Aliquots of standard working solution of different concentration in the range 200-1200ng/spot of brivaracetam
were spotted on the plate. The calibration curve was plotted peak area vs concentration by using Win-CATS software. After 20min chamber saturation time was over the plate was developed in twin trough glass chamber. The length of the run was 80mm. The experiment was repeated three times to generate the calibration equation and then least square regression analysis was performed.

**Precision**

To evaluate intra-day precision, samples were analyzed on same day. Three different samples of three concentrations(200ng/spot, 400ng/spot and 800ng/spot) were analyzed. The inter-day precision study was carried out by comparing the data of three different days.

**Repeatability**

Repeatability of sample application was ensured by spotting six spots of 600ng/spot standard drug solution on a TLC plate at different times on same day with the help of sample applicator, followed by development of plate and recording of the peak areas for six spots.

**Accuracy**

For determining accuracy of the developed method recovery studies of the drug were carried out. Accuracy was assessed by mixing known quantity of standard drug ‘Briviact’ which is manufactured by Sanofi India, and the contents were analyzed by the standard method. Recovery studies were carried out at 80-120% levels. Percentage recovery and percentage RSD were then calculated.

**Limit of detection and limit of quantitation**

To calculate the limit of detection (LOD) and limit of quantitation (LOQ) methanol was spotted six times. LOD studies were carried out by taking different concentrations as 20, 40, 60, 80, 100ng/spot. Spot was not detected up to concentration 40ng/spot. The peak was observed at 60ng/spot and a signal-to-noise ratio is 3:1. The LOQ was performed by taking different concentrations as 20, 40, 60, 80, 100ng/spot. The peak was observed with quantifiable area at 600ng/spot with a signal-to-noise ratio of 10:1.

**Specificity**

To confirm the specificity, brivaracetam was spotted on TLC plate, developed and scanned. The standard UV spectrum of brivaracetam was compared with spectrum of brivaracetam extracted from tablet. The peak purity of brivaracetam was assessed by comparing their respective spectra at different position of spot.

**Robustness**

For the study of robustness various parameters were selected viz. mobile phase composition, chamber saturation time and solvent migration distance. The small change in one factor was introduced and the effect on the results was examined.

**Result and discussion**

**Optimization of mobile phase**

The most suitable mobile phase is ammonium acetate: methanol: n-propanol (8:1.6:1.6 v/v/v). It gave better resolution and better reproducibility in migration of brivaracetam [Fig.2].
### Table 1: Forced Degradation Studies

<table>
<thead>
<tr>
<th>Forced degradation condition</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value of degradation products</th>
<th>% Degradation</th>
<th>% Drug</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid, 0.01 N HCl, 50⁰C for 15 min</td>
<td>Peak 1- 0.03, Peak 2- 0.47</td>
<td>6.1±0.29</td>
<td>96.9±0.24</td>
<td>Fig.3 A</td>
</tr>
<tr>
<td>Alkaline 0.01 N NaOH, 50⁰C for 15 min</td>
<td>Peak 1- 0.06, Peak 2- 0.47, Peak 3- 0.84</td>
<td>8.8±1.12</td>
<td>91.2±1.01</td>
<td>Fig.3 B</td>
</tr>
<tr>
<td>H₂O₂ 3%w/v, 2 hrs</td>
<td>Peak 1- 0.06, Peak 2- 0.47, Peak 3- 0.62, Peak 4- 0.64</td>
<td>12.9±2.7</td>
<td>90.1±2.15</td>
<td>Fig.3 C</td>
</tr>
</tbody>
</table>

**Figure 2: Densitogram of brivaracetam formulation (800 ng/spot)**

A. Densitogram of Acid Degradation  
B. Densitogram of Base Degradation
A forced degradation study was carried out. Degradation was observed in acid, alkaline and oxidative conditions. The drug was stable in photochemical studies.\[Fig.3\] \[Table 1\]

**Method validation**

**Linearity**

The calibration curve was plotted as peak area of compound against the concentration over the range of 200 to 1200ng/spot. The slope, intercept and correlation co-efficient value was calculated and it is given in \[Table 2\]. Graph showed that their exhibits a good correlation between regression coefficient and concentration of the drug \[Fig.4\].

\[B- Acid induced Degradation, B- Base Induced Degradation, C- Oxidative Degradation\]
Table 2: Linearity and range

<table>
<thead>
<tr>
<th>Linearity and range</th>
<th>Brivaracetam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (ng/spot)</td>
<td>200-1200</td>
</tr>
<tr>
<td>Regression coefficient ($r^2$)</td>
<td>0.997</td>
</tr>
<tr>
<td>Linearity equation</td>
<td>$y = 3.793x - 43.7$</td>
</tr>
</tbody>
</table>

### Precision

The intra-day and inter-day was performed and relative standard derivations were found in the range of 0.54-0.87% and 0.25-0.46% respectively. Smaller the values of intra-day and inter-day variation in the analysis indicated that the method was precise. [Table 3]

Table 3: Intra-day and Inter-day precision study (n=3)

<table>
<thead>
<tr>
<th>Amount (ng/spot)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area</td>
<td>%RSD</td>
</tr>
<tr>
<td>40</td>
<td>1617</td>
<td>0.8749</td>
</tr>
<tr>
<td>60</td>
<td>2271</td>
<td>0.5468</td>
</tr>
<tr>
<td>80</td>
<td>2952</td>
<td>0.5420</td>
</tr>
</tbody>
</table>

Table 4: Repeatability study

<table>
<thead>
<tr>
<th>Amount (ng/spot)</th>
<th>Peak area</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2214</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2244</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2246</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2235</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2246</td>
<td>0.5989</td>
</tr>
</tbody>
</table>

### Repeatability

In repeatability study of sample application, the %RSD for the peak area of given sample was found to be 0.59%. The RSD value is for the measurement of peak area and sample application, both of these are the instrumental specifications (i.e. 1%); which ensure the proper functioning of the system. [Table 4]

### Accuracy

The % recovery of brivaracetam was found to be 97.53, 99.86, and 102.84% (at 80%, 100% and 120% respectively). The recovery studies results indicated that the given method was accurate for estimation of drug in a tablet dosage form [Table 5].

Table 5: Recovery studies of brivaracetam tablet (n=3)

<table>
<thead>
<tr>
<th>% Level</th>
<th>Concentration of drug added ng/spot</th>
<th>Concentration of drug found</th>
<th>% Recovery</th>
<th>Average Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>324</td>
<td>316</td>
<td>97.53</td>
<td>100.07</td>
</tr>
<tr>
<td>100</td>
<td>360</td>
<td>359</td>
<td>99.86</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>396</td>
<td>407</td>
<td>102.84</td>
<td></td>
</tr>
</tbody>
</table>

### LOD and LOQ

The LOD gives a measurable response of signal to noise ratio of 3:1. The LOD for brivaracetam found to be 60ng/spot. The LOQ gives response that can be accurately quantified (signal to noise ratio of 10:1). The LOQ was found to be at 600ng/spot for brivaracetam. Therefore it was conclude that the developed method was sensitive.
Analysis of formulation

The content of the brivaracetam tablet (Sanofi India, Powai, Mumbai) was calculated from the peak area which were recorded from densitogram. Analysis of brivaracetam(25mg)tablet was performed and % label claim was found to be 98.64%. [Fig.5] [Table 6]

Table 6: Analysis of formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount (µg/tablet)(n*=3)</th>
<th>% Label claim</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brivaracetam</td>
<td>360 Added(ng) 355 Found(ng)</td>
<td>98.64</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*n= no of times procedure repeated

Figure 5: Densitogram of brivaracetam tablet sample

Figure 6: UV spectrum of standard brivaracetam
Specificity

Specificity is the method for analyzing Rf value and spectra pattern of drug. The good correlation among spectra gives the peaks at start (s), apex (m), and end (e) indicates the peak purity of brivaracetam [correlation \( r(s, m) = 0.20, 0.26, r(m, e) = 0.26, 0.31 \)]. Therefore it can be assured that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drug. It was observed that excipients present in formulation did not interfere with peak of drug (\( R_f 0.40\pm0.03 \)). Hence, above HPTLC method was found to be specific. [Fig.6]

Robustness

Robustness is the effect of operational parameters examination on the analysis results. Small changes in mobile phase composition were introduced and deviation was observed. 2% RSD deviation was observed which confirmed the proposed method is robust.

Conclusion

The present research work gives satisfactory result for method validation and development of stability indicating HPTLC method for quantitative determination of brivaracetam. The HPTLC method is simple, rapid, economic and more flexible for routine analysis of brivaracetam in bulk and tablet dosage forms. Thus, the proposed method is of considerable importance and has sound industrial applicability for quality control and analysis of brivaracetam from bulk drug and formulations.

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Conflict of Interest

The authors have no conflict of interest to declare.

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