QUANTIFICATION OF PREGABALIN AND ETORICOXIB COMBO IN TABLETS AND BULK WITH DEVELOPED RP-HPLC METHOD: STABILITY INDICATING FEATURE ASSESSMENT

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ABSTRACT
This investigation reports a stability indicating HPLC method to quantify Pregabalin (PGBN) and etoricoxib (ERCB) in tablets form and bulk form. The PGBN and ERCB were quantified on Thermo C18 column with Orthophosphoric acid (0.1%), and methanol 60:40 vol/vol ratio was employed as mobile phase. The amounts of PGBN and ERCB were enumerated by detector fixed at 236 nm. The procedure demonstrated reasonable linearity for PGBN with R² = 0.9998 in the quantity scale 37.5 to 112.5 µg/ml, and for ERCB with R² = 0.9999 in the quantity scale 30 to 90 µg/ml. The LOD as well as LOQ for PGBN are 0.201µg/ml & 0.670 µg/ml and for ERCB are 0.106 µg/ml & 0.355 µg/ml. The RT’s for PGBN was 2.636 min and ERCB was 5.607 min and whole analysis time was 8 min. Stability degradation experiments were used to establish the method's capacity to indicate stability indicating. The conditions include acid forced hydrolysis, thermal forced degradation, alkali forced hydrolysis, oxidative forced degradation and photo forced degradation conditions. Detection as well as quantification of PGBN and ERCB was unaffected by the degradants.

Keywords: Stability indicating, Pregabalin, RP-HPLC, Etoricoxib, Evaluation.

1. INTRODUCTION
Pregabalin (PGBN, Fig. 1) is recommended to relieve neuropathic pains allied with spinal cord injury, peripheral neuropathy in diabetics, fibromyalgia and postherpetic neuralgia [1, 2]. PGBN has also received reorganization by FDA as an adjunct medication for people diagnosed epilepsy that is enduring partial-onset episodes [3]. Off-label PGBN practices include general anxiety disorder, bipolar disorder, social anxiety disorder, chronic pain and insomnia conditions not else permitted by FDA [4]. Within tissues in central nervous system, PGBN connects to presynaptic voltage-fenced Ca²⁺ channels at the α-2-δ subunit. Binding of the α-2-δ subunit inhibits the release of excitatory neurotransmitters and diminishes the depolarization-persuaded Ca²⁺ influx into neurons [5]. The analgesic as well as anticonvulsant properties of PGBN might be attributed to above mechanism.

Upon comparison to typical nonsteroidal anti-inflammatory medications, etoricoxib (ERCB, Fig. 1) is a specific cyclooxygenase-2 antagonist with a lesser risk of gastrointestinal complications [6].

Fig. 1: Drug structures

With ERCB, reductions in painful as well as swollen joint scores were much larger in subjects experiencing rheumatoid arthritis. ERCB was considerably more...
successful in improving pain and physical function ratings in subjects experiencing osteoarthritis of the knee and hip [7]. Subjects experiencing postoperative oral discomfort were alleviated by a single dosage of ERCB [8]. In individuals with persistent low back pain, ERCB provided much superior pain alleviation. In patients experiencing acute gout and women experiencing primary dysmenorrhea, ERCB demonstrated equal effectiveness [9, 10].

For neuropathic discomfort, a combo of ERCB and PGBN is better effective over monotherapy. ERCB and PGBN combo quantification was made by HPLC [11, 12] and UV spectrophotometry [13] methods. HPLC method by Amit and Bhuvanesh [11] includes three solvents as mobile phase, increasing the solvent use and analytical costs. HPLC method by Upeksha and Tarai takes total analysis run spell of 9 min. HPLC procedures of Amit & Bhuvanesh [11] and Upeksha & Tarai [12], and spectrophotometry procedure of Prakash et al., [13] reported LOQ of greater than 1 µg/ml. None of the researchers mentioned the forced degradation research on combo of ERCB and PGBN [11-13]. Furthermore, none of the published approaches indicated that they were stability indicating.

We intended to design an RP-HPLC technology that used two solvents for the mobile phase, had a shorter overall analytical run time, and had a LOQ of smaller than 1 µg/ml. We have done forced degradation research on combo of ERCB and PGBN and also gauged stability indicating characteristic nature.

2. MATERIAL AND METHODS

2.1. Instruments
The chromatographic assessment for the combination of ERCB and PGBN was performed that use a "Waters HPLC system, 2695 series" with a "Waters quaternary pump" and a "Waters thermostatic column compartment" that controlled 25°C of column temperature at 25°C. An "Waters autosampler" was used to introduce ERCB and PGBN combo samples. An "Waters photodiode detector, 2998 series" was coupled to the HPLC device. The chromatograms of ERCB and PGBN combo were recorded and interpreted using "Waters programme Empower2."

2.2. Material
ECRB standard (99.6% purity) and PGBN standard (99.9% purity) was liberally offered by the "SRC Laboratories Pvt Ltd, Karnataka, India". Emaxgalin tablets (PGBN - 75mg and ECRB - 60mg) produced by “Sun Pharmaceutical Industries Ltd, India” were obtained from the Indian market. "Merck India Ltd, India" supplied the methanol. From "Sd-Fine Chemicals Ltd, India", acquired sodium hydroxide, hydrogen peroxide, phosphoric acid and hydrochloric acid solutions.

2.3. Standard solutions
Stock PGBN and ERCB solution (PGBN - 750 µg/ml and ECRB - 600µg/ml) were made in mobile phase (phosphoric acid: methanol mixture with 60:40 ratio volumes). Working PGBN and ERCB solutions (PGBN - 750 µg/ml and ECRB - 600µg/ml) were also made ready in mobile phase.

2.4. Procedure
Chromatographic separations of PGBN and ERCB combo were achieved in our investigation employing an isocratic technique adopting a C18 column, “Thermo, 250 mm × 4.6 mm, 5 µm”. Phosphoric acid: methanol mixture (pH 5.0) with 60:40 ratio volumes at a persistent flow of 1 ml/min was the mobile phase. The eluates (PGBN and ERCB) were investigated at a 236 nm wavelength. Each infusion run lasting 8 min, with a 10 µl infusion volume. The overall peak areas of the examined drugs (PGBN and ERCB) were applied to quantify the PGBN and ERCB contents.

2.5. Linearity curves
Appropriate aliquots corresponding to 37.5 to 112.5 µg/ml of PGBN and 30 to 90 µg/ml of ERCB were transferred from the stock PGBN and ERCB solution (PGBN - 750 g/ml and ECRB - 600 g/ml) to multiple sets of 10 ml gauging flasks and adjusted to marking with mobile phase. 10 µl injections of each concentration were carried out. The obtained solutions were filtered before being diluted further with the chromatographic mobile phase to

2.6. Application to Emaxgalin tablets
By weighing and grinding 10 Emaxgalin pills, a fine powder was achieved. A quantity of powder having 75 mg PGBN and 60 mg ECRB was precisely weighed and placed into a graduated flask holding 60 ml mobile phase. The solution mix then was sonicated for 30 min, cooled till room temperature, and afterwards filled to an ultimate volume of 100 ml using mobile phase. The obtained solutions were filtered before being diluted further with the chromatographic mobile phase to
achieve a standard Emaxgalin solution concentration with 75 µg/ml PGBN and 60 µg/ml ECRB. The full approach under "2.4. Procedure" and "2.5. Linearity curves" was followed, and the concentrations of PGBN and ECRB were obtained utilizing the regression equation, as well as the recovery percent of PGBN and ECRB were computed.

2.7. Stability tests
Stability experiments for the PGBN and ECRB products (Emaxgalin tablets) were carried out under a variety of stress instances as specified by the ICH recommendations [14]. For this purpose, the samples (10 ml) were exposed to acid forced hydrolysis (10 ml, 0.1 N HCl, 30 min, sonication, near 25°C), thermal forced degradation (near 60°C in oven for 30 min), alkali forced hydrolysis (10 ml, 0.1 N NaOH, 30 min, sonication, near 25°C), oxidative forced degradation (10 ml, 30% peroxide, 30 min, sonication, near 25°C) and photo forced degradation (sun light, 6 hr) conditions.

3. RESULTS AND DISCUSSION
3.1. PGBN and ECRB assaying method development
Several factors that influence chromatographic PGBN and ECRB separation were assessed then optimised. These characteristics involved scanning of diverse wavelengths (200-400 nm), experimenting with varied aqueous phase types (0.1 M KH₂PO₄, 0.1 M K₂HPO₄ and 0.1% phosphoric acid), different categories besides ratios of organic modifier (acetonitrile and methanol) added. High sensitivity through negligible detected noise, better resolution of PGBN & ECRB, good PGBN & ECRB peak symmetries and reasonably less RT’s for PGBN & ECRB were obtained with employing an isocratic technique adopting a C18 column, “Thermo, 250 mm × 4.6 mm, 5 µm”, Phosphoric acid: methanol mixture (pH 5.0) with 60:40 ratio volumes at a persistent flow of 1 ml/min was the mobile phase, and eluates (PGBN and ECRB) were investigated at a 236 nm wavelength. The RT’s for PGBN was 2.636 min and ECRB was 5.607 min (Fig. 2).

3.2. Validation
Validation of developed PGBN and ECRB combination assaying approach was made as specified by the ICH recommendations [15].

3.2.1. Linearity
The linearity range of PGBN and ECRB combo assaying methodology was documented as 37.5 to 112.5µg/ml for PGBN and 30 to 90 µg/ml for ECRB. The regression equation for PGBN in mobile phase was perceived as $P = 18086 \cdot c - 7041.2$, and for ECRB, it was perceived as $P = 74216 \cdot c - 24058$. Where ‘P’ is PGBN/ECRB peak area and “c” is PGBN/ECRB concentration. The computed correlation coefficients for PGBN and ECRB were 0.9998 and 0.9999, respectively. The developed PGBN and ECRB combination assaying approach was evidenced to be linear based on those findings.

3.2.2. LOD and LOQ
In compared to blank diluent, the concentration of PGBN and ECRB was deemed LOD, with a signal-to-noise proportion of ≥3. Likewise, in compared to blank diluent, the concentration of PGBN and ECRB was deemed LOQ, with a signal-to-noise proportion of ≥10. PGBN’s measures were 0.201µg/ml (LOD) and 0.670 µg/ml (LOQ). ECRB’s measures were 0.106µg/ml (LOD) and 0.355µg/ml (LOQ).

Fig. 2: Typical PGBN and ECRB chromatogram

![Typical PGBN and ECRB chromatogram](image-url)
3.2.3. Precision
The precision of PGBN and ECRB combination assaying approach were computed by studying % RSD of PGBN and ECRB peak areas at concentration 75µg/ml (PGBN) and 60µg/ml (ECRB). % RSD of PGBN and ECRB peak areas were assessed as 0.297% with SD of 3999.620 and 0.149% with SD of 6608.162, respectively, indicated that the developed PGBN and ECRB combination assaying approach was precise.

3.2.4. Accuracy
The accuracy of PGBN and ECRB combination assaying approach were computed by studying % recovery of PGBN and ECRB peak areas at concentration 75µg/ml (PGBN) and 60µg/ml (ECRB). The percentile recovery of PGBN and ECRB peak areas was reported to be 98.547% with SD of 0.292 and 99.317 with SD of 0.148, respectively, indicating that the established PGBN and ECRB combination assaying technique appeared accurate.

3.2.5. Selectivity
The selectivity of the PGBN and ECRB combination assaying approach was obtained in emaxgalin solution (75 µg/ml PGBN and 60 µg/ml ECRB) added at three different concentrations with reference PGBN and ECRB, namely, 50% (37.125 µg/ml PGBN and 29.70 µg/ml ECRB), 100% (74.250 µg/ml PGBN and 59.40 µg/ml ECRB), and 150% (111.375 µg/ml PGBN and 89.10 µg/ml ECRB). At three different PGBN and ECRB concentrations, recoveries ranged from 99.68% to 100.69% (Table 1). The significant percent recoveries demonstrated that the technique was quite selective for estimating PGBN and ECRB in emaxgalin tablets despite interruption from excipients.

3.2.6. Robustness
The impact of methanol ratio composition (±5%), pH (±0.1), temperature (±5 degree Celsius) and flow rate (±0.1) minute changes on PGBN and ECRB combination assaying technique performance were studied. The % RSD of PGBN and ECRB peak areas at concentration 75 µg/ml (PGBN) and 60 µg/ml (ECRB) for PGBN and ECRB assay robustness were calculated and verified to be inside acceptable norms (Table 2). The results of this investigative process suggested that the designed PGBN and ECRB combination assaying technique was robust.

### Table 1: Selectivity results for estimating PGBN and ECRB in emaxgalin tablets

<table>
<thead>
<tr>
<th>Added µg/ml</th>
<th>PGBN Found</th>
<th>Percentile Recovery</th>
<th>Mean</th>
<th>ECRB Found</th>
<th>Percentile Recovery</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.125</td>
<td>37.34</td>
<td>100.59</td>
<td>100.69</td>
<td>29.700</td>
<td>99.53</td>
<td>99.99</td>
</tr>
<tr>
<td>74.250</td>
<td>74.49</td>
<td>100.32</td>
<td>100.28</td>
<td>89.100</td>
<td>100.32</td>
<td>100.37</td>
</tr>
<tr>
<td>111.375</td>
<td>112.11</td>
<td>100.66</td>
<td>100.48</td>
<td>89.38</td>
<td>100.32</td>
<td>100.48</td>
</tr>
</tbody>
</table>

### Table 2: PGBN and ECRB combination assaying technique robustness

<table>
<thead>
<tr>
<th>Alternation in</th>
<th>Peak area*</th>
<th>RSD</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol composition</td>
<td>4504273.0</td>
<td>1.8</td>
<td>82520.5</td>
</tr>
<tr>
<td>Flow rate</td>
<td>4586462.3</td>
<td>1.6</td>
<td>74828.3</td>
</tr>
<tr>
<td>Detection nm</td>
<td>4578692.0</td>
<td>1.8</td>
<td>80737.0</td>
</tr>
<tr>
<td>pH</td>
<td>4528020.3</td>
<td>1.2</td>
<td>56405.1</td>
</tr>
<tr>
<td>Temperature</td>
<td>4578366.7</td>
<td>1.8</td>
<td>80694.9</td>
</tr>
</tbody>
</table>

*mean - three determinations
3.2.7. PGBN and ECRB stability

The PGBN and ECRB stabilities after exposure to acid forced hydrolysis, thermal forced degradation, alkali forced hydrolysis, oxidative forced degradation and photo forced degradation conditions were summarized in Table 3. In heat forced degradation, PGBN and ECRB are the least stable. In alkali forced hydrolysis, PGBN seems to be more stable, but ECRB seems to be more stable with oxidative forced degradation.

3.2.8. Stability indicating and Specificity

The PGBN and ECRB chromatograms after exposure to acid forced hydrolysis, thermal forced degradation, alkali forced hydrolysis, oxidative forced degradation and photo forced degradation conditions were given in Fig. 3. The extra peaks in chromatograms characterize the degradation of PGBN and ECRB and formation of PGBN and ECRB degradation possible products. The chromatograms have proven the feature, stability indicating, as peaks of degradation possible products are satisfactory separated from PGBN and ECRB peaks. There are none coeluting peaks during the retention period of PGBN and ECRB, as can be shown from the peak purity assessment (Table 4). This result corroborated the specificity by indicating that the PGBN and ECRB peak was pure.

Table 3: PGBN and ECRB stabilities

<table>
<thead>
<tr>
<th>Conditions</th>
<th>PGBN</th>
<th>ECRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Stability</td>
<td>% Instability</td>
<td>% Stability</td>
</tr>
<tr>
<td>Acid forced hydrolysis</td>
<td>91.48</td>
<td>8.52</td>
</tr>
<tr>
<td>Alkali forced hydrolysis</td>
<td>93.37</td>
<td>6.63</td>
</tr>
<tr>
<td>Oxidative forced degradation</td>
<td>92.79</td>
<td>7.21</td>
</tr>
<tr>
<td>Thermal forced degradation</td>
<td>88.51</td>
<td>11.49</td>
</tr>
<tr>
<td>Photo forced degradation</td>
<td>90.79</td>
<td>9.21</td>
</tr>
</tbody>
</table>

Fig. 3: Stability indicating associated chromatograms
Table 4: Assessment of peak purities of PGBN and ECRB

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drug</th>
<th>Purity angle</th>
<th>Purity threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid forced hydrolysis</td>
<td>PGBN</td>
<td>0.216</td>
<td>0.870</td>
</tr>
<tr>
<td></td>
<td>ECRB</td>
<td>0.342</td>
<td>0.611</td>
</tr>
<tr>
<td>Alkali forced hydrolysis</td>
<td>PGBN</td>
<td>0.291</td>
<td>0.768</td>
</tr>
<tr>
<td></td>
<td>ECRB</td>
<td>0.342</td>
<td>0.606</td>
</tr>
<tr>
<td>Oxidative forced degradation</td>
<td>PGBN</td>
<td>0.381</td>
<td>0.670</td>
</tr>
<tr>
<td></td>
<td>ECRB</td>
<td>0.412</td>
<td>0.802</td>
</tr>
<tr>
<td>Thermal forced degradation</td>
<td>PGBN</td>
<td>0.278</td>
<td>0.770</td>
</tr>
<tr>
<td></td>
<td>ECRB</td>
<td>0.325</td>
<td>0.504</td>
</tr>
<tr>
<td>Photo forced degradation</td>
<td>PGBN</td>
<td>0.399</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>ECRB</td>
<td>0.248</td>
<td>0.507</td>
</tr>
</tbody>
</table>

4. CONCLUSION

We designed an RP-HPLC technology for combo analysis of ERCB and PGBN in bulk and emaxgalin tablets. The RP-HPLC technology for combo analysis had advantages like: used two solvents for the mobile phase, had a shorter overall analytical run time, and had a LOQ of smaller than 1 µg/ml. The forced degradation research also done on combo of ERCB and PGBN to assess their stabilities in different acid forced hydrolysis, thermal forced degradation, alkali forced hydrolysis, oxidative forced degradation and photo forced degradation conditions. The method, moreover, proved as having stability indicating characteristic nature, preciseness and accurateness.

Conflict of interest
None declared

Source of funding
None declared

5. REFERENCES

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