Development and validation of colorimetric method for the determination of pregabalin in bulk and pharmaceutical formulations

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ABSTRACT

A simple, accurate and precise colorimetric method was developed for the quantification of pregabalin (PGN) in bulk and pharmaceutical formulations. The method was based on the production of a colored complex by reacting PGN with ascorbic acid in DMSO solvent. The resultant complex exhibits absorption maxima at 390 and 532 nm. The factors that affect the complex development and stability were studied and optimized. The reaction stoichiometry of drug: reagent was found to be 1:2. The method was then validated according to ICH guidelines. Regression analysis of Beer’s plot showed good correlation (r² value more than 0.998) within a concentration range of 5-30 µg/ml. Linearity was confirmed by standardized residuals versus fitted value plots using Minitab14 software. The limits of detection and quantification at 390 and 532 nm were 1.34 µg/ml, 1.09 µg/ml and 4.05 µg/ml, respectively. Added recovery measurements were found to be 100.04 ± 2.07% and 99.58 ± 1.98% at 390 nm and 532 nm, respectively, which reflect the accuracy of the method and freedom from interference (relative standard deviation values were less than or equal 0.2%). The average assay for the commercial capsule preparation (PGN 150 mg/capsule) was found to be 101.37 ± 0.49 and 101.81 ± 0.73 at 390 and 532 nm, respectively. The developed method is an inexpensive, extraction free and can be applied for routine analysis of PGN in most of analytical laboratories.

INTRODUCTION

PGN (Figure 1) is an antiepileptic amino acid used for treatment of partial seizure and neuropathic pain. It is a GABA (gamma amino butyric acid) analog, works by binding to the voltage-gated Ca²⁺ channels (Katzung et al., 2012). It has a simple aliphatic structure with no chromophore, hence exhibits weak UV absorbance making spectrophotometric determination of the drug somehow difficult. Many spectrophotometric (Shep and Lahoti, 2013), (Patel et al., 2016) spectrofluorimetric (Derayea et al., 2018), fluorometric (Yoshikawa et al., 2016) and chromatographic (HPLC) (Akther et al., 2013) methods have been reported in the literature.
analytical reagent in pharmaceutical analysis. It is known to have a selective reaction with ammonia and primary aliphatic amines of the type R-CH$_2$-NH$_2$ ($\lambda_{max}$ 390 and 530 nm) (Pesez and Bartos, 1974). This reagent was reported to be used in the determination of various drugs including lisinopril (Rahman et al., 2005), paromomycin (Adam et al., 2016b), gabapentin (Adam et al., 2016a), and tranexamic acid (Gadkariem et al., 2013). Most of the reported methods for PGN analysis need highly trained personnel, expensive or unavailable equipment or reagents. Based on these reports, simple, precise, accurate and inexpensive colorimetric method was developed for the determination of PGN in bulk and capsule forms using ascorbic acid as a chromogen.

MATERIALS AND METHODS

All materials and reagents used were of analytical grade. PGN authentic standard (M.wt 159.2 and 99% claimed purity) was kindly provided by Pharmaland Pharmaceuticals, Sudan. Sample Z of PGN capsules (150mg) was obtained from the local market. Ascorbic acid, Dimethylsulfoxide (DMSO) and Dimethylformamide (DMF) were purchased from S.D Fine chem. Limited, India.

Spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, Kyoto, Japan.

Preparation of stock solutions

Standard stock solution

PGN standard (100mg) was accurately weighed and transferred into a 10 ml volumetric flask. 7 ml of distilled water were added and the solution was sonicated for 30 min. to ensure dissolution. The volume was then completed to the mark with distilled water (Solution A; 1%w/v; 10mg/ml).

Sample stock solution

Out of 20 capsules of sample Z, an amount equivalent to 100mg of PGN was accurately weighed and treated as PGN standard (Solutions B; 1%w/v; 10mg/ml).

Ascorbic acid solution

Fifty milligrams of ascorbic acid was accurately weighed and transferred into 25 ml volumetric flask. About 20 ml of DMSO were added and the solution was shaken for 5 minutes to ensure dissolution. The volume was completed to the mark with DMSO and mixed well (0.2%w/v).

Reagent blank

About 100 $\mu$l of distilled water were transferred into a 10 ml volumetric flask. 1 ml of freshly prepared 2% w/v ascorbic acid solution was added and the volume was made up to the mark with DMSO.

Procedure

Optimization
To determine the optimum solvent, one ml of ascorbic acid solution was transferred into a 10 ml volumetric flask containing 40 \( \mu l \) of solution A, the volume was made up to the mark with DMSO and heated for 30 min. then cooled and scanned against blank solutions. (The same experiment was carried out with DMF)

![Figure 4: Residual plots of PGN ascorbic acid complex](image)

![Figure 5: Molar ratio of PGN reaction with ascorbic acid (drug to reagent)](image)

In order to get the best heating time, one ml of ascorbic acid solution was added to a set of volumetric flasks containing 60 \( \mu l \) of solution A. The solutions were treated as above and heated in a boiling water bath for 10, 20, 30 & 40 min., respectively. After cooling the solutions were scanned against blank solutions.

**Linearity**

Serial volumes of solution A (5-30 \( \mu l \)) were transferred into a set of 10 ml volumetric flasks. Distilled water was added to each flask to obtain 100 \( \mu l \). 1 ml of freshly prepared ascorbic acid solution (2%) solution was added to each flask and the volume was made up to the mark with DMSO. The solutions were transferred into stoppered glass test tubes and heated for 30 min. in a boiling water bath. After cooling to room temperature, the absorbance of each solution was measured against reagent blank at 390nm and 532nm. Absorbance values obtained were plotted against PGN concentration values and the calibration curves at 390nm and 532nm were constructed. Linearity was confirmed using Minitab 14 software to produce standardized residual versus fitted values plot.

**Assay**

Serial dilutions of solutions B were treated as under calibration curve. The content of capsules was determined by direct sample/standard comparison.

**Added recovery**

Ten microlitre of solution A and B were transferred into two separate 10 ml volumetric flasks. Ten microlitre of solutions A and B were mixed in a third one and all three were treated as under calibration curve (100% level). The process was repeated at 50% and 150% levels. Percent added recovery was calculated as follows:

\[
R\% = \frac{Mixture \text{ response} - \text{Sample response}}{\text{Standard response}} \times 100
\]

**Standard addition**

Zero, 10, 20 and 30 \( \mu l \) of solution A were transferred into separate 10 ml volumetric flasks. 10 \( \mu l \) of solution B was added to each and treated as under calibration curve. The concentration of the first solution (X) is then calculated by taking Y = zero in the calibration curve equation and represented as a percent of the added amount.

**Stoichiometry (molar ratio method)**

Serial volumes (5, 10, 15, 20, 25, 30, 35 & 40\( \mu l \)) of PGN solution (5.0 \( \times 10^{-2} \) M) were transferred into 10 ml volumetric flasks and the volume of each was made up to 100 \( \mu l \) with distilled water. 20 \( \mu l \) of freshly prepared ascorbic acid solution (5.0 \( \times 10^{-2} \) M solution) was added to each and the solutions were treated as under calibration curve.

The molar ratio of the reaction was obtained from a plot of concentration ratio ([PGN]/[ascorbic acid]) versus absorbance values.

**RESULTS AND DISCUSSION**

PGN is a simple aliphatic amino acid with weak UV absorbance (210nm) (Shep and Lahoti, 2013). This absorbance is highly susceptible to overlapping with most solvents as they absorb at the same UV region. Reaction with a suitable chromogen can produce a chromophore enabling the analysis of PGN by spectrophotometry. Ascorbic acid was found to react with PGN in DMSO, giving a purple-coloured complex with two \( \lambda_{max} \) (390nm and 532nm) suggesting two transitions. Experimental factors affecting the colour development, intensity and stability were studied and optimized to produce the spectrum presented in Figure 2. DMSO was found to give better
**Table 1: Comparison between DMSO & DMFA regarding absorption intensity and $\lambda_{\text{max}}$**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$</th>
<th>Absorbance</th>
<th>$\lambda_{\text{max}}$</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>532nm</td>
<td>0.439</td>
<td>390nm</td>
<td>1.318</td>
</tr>
<tr>
<td>DMF</td>
<td>528nm</td>
<td>0.140</td>
<td>384nm</td>
<td>0.508</td>
</tr>
</tbody>
</table>

**Table 2: Absorbance values of PGN-ascorbic acid complex at different heating times**

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Absorbance 390 nm</th>
<th>Absorbance 532 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.394</td>
<td>0.477</td>
</tr>
<tr>
<td>20</td>
<td>1.651</td>
<td>0.557</td>
</tr>
<tr>
<td>30</td>
<td>1.772</td>
<td>0.579</td>
</tr>
<tr>
<td>40</td>
<td>1.805</td>
<td>0.606</td>
</tr>
</tbody>
</table>

**Table 3: Absorbance values of PGN-ascorbic acid complex with different reagent concentrations**

<table>
<thead>
<tr>
<th>Reagent concentration (%w/v)</th>
<th>Absorbance 390 nm</th>
<th>Absorbance 532 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.373</td>
<td>0.134</td>
</tr>
<tr>
<td>0.1</td>
<td>1.002</td>
<td>0.336</td>
</tr>
<tr>
<td>0.2</td>
<td>1.733</td>
<td>0.593</td>
</tr>
<tr>
<td>0.3</td>
<td>1.684</td>
<td>0.578</td>
</tr>
<tr>
<td>0.4</td>
<td>0.593</td>
<td>0.536</td>
</tr>
</tbody>
</table>

**Table 4: Linearity data of the developed method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values 390nm</th>
<th>Values 532nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range ($\mu$g/ml)</td>
<td>5-30</td>
<td>5-30</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>0.99897</td>
<td>0.9988</td>
</tr>
<tr>
<td>Intercept ± SE*</td>
<td>0.0615 ± 0.0217</td>
<td>0.0238 ± 0.006</td>
</tr>
<tr>
<td>Slope ± SE*</td>
<td>0.0575 ± 0.0011</td>
<td>0.0197 ± 0.0003</td>
</tr>
<tr>
<td>LOD ($\mu$g/ml)</td>
<td>1.34</td>
<td>1.09</td>
</tr>
<tr>
<td>LOQ ($\mu$g/ml)</td>
<td>4.05</td>
<td>3.294</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>9717.4</td>
<td>3356</td>
</tr>
</tbody>
</table>

**Table 5: percent recovery results of sample Z at 50,100 and 150 percent levels**

<table>
<thead>
<tr>
<th>Level (%)</th>
<th>$\lambda_{\text{max}}$</th>
<th>Percent recovery ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>390nm</td>
<td>100.78±2.00</td>
</tr>
<tr>
<td></td>
<td>532nm</td>
<td>101.64±0.79</td>
</tr>
<tr>
<td>100</td>
<td>390nm</td>
<td>103.22±1.33</td>
</tr>
<tr>
<td></td>
<td>532nm</td>
<td>100.54±1.08</td>
</tr>
<tr>
<td>150</td>
<td>390nm</td>
<td>99.95±2.07</td>
</tr>
<tr>
<td></td>
<td>532nm</td>
<td>99.57±1.98</td>
</tr>
</tbody>
</table>

**Table 6: assay values of sample Z by direct sample/standard comparison**

<table>
<thead>
<tr>
<th>$\lambda_{\text{max}}$</th>
<th>True mean</th>
<th>Sample Z Content*</th>
<th>t- calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>390nm</td>
<td>100</td>
<td>101.373±0.49</td>
<td>1.947</td>
</tr>
<tr>
<td>532nm</td>
<td>100</td>
<td>101.812±0.73</td>
<td>1.963</td>
</tr>
</tbody>
</table>
peak shapes and more intense absorbance than DMF (Table 1).

Heating to 100 °C was found necessary for colour development. The most available maximum of absorbance was reached with heating for 30 minutes (Table 2).

The highest absorbance levels were observed with 1 ml of 0.2% ascorbic acid solution (Table 3). The developed colour was found stable for more than 180 minutes after which the absorbance intensity decreased very slightly with passage of time.

**Linearity**

The constructed calibration curves (Figure 3) obeyed Beer's law over the concentration range 5–30 μg/ml. The linearity data was calculated at 95% confidence limit and summarized in Table 4. The two plots in Figure 4 shows a narrow range of magnitude difference between the standardized residuals and the fitted values of data points. This reflects the closeness of actual points to the best-fitted line and confirms the linearity of the regression curve. Additionally, the normal scattering of data points above and below the zero line means there is no systematic error.

**Accuracy and precision**

Accuracy of the developed method was tested by recovery percent and standard addition methods. The obtained results of the percent recovery of sample Z are summarized in Table 5. Standard addition test results were (93% and 96%; at 390nm and 532nm, respectively. The obtained results reflected the freedom from any interference.

The developed method was then applied for the assay of pharmaceutical formulation. Results are summarized in Table 6. Statistical comparison was conducted using the following formula:

\[ t = \frac{(\bar{x} - \mu) \sqrt{n}}{s} \]

where \( \bar{x} \) is the calculated mean, \( \mu \) is the true mean and \( s \) is the standard deviation.

It was found that there are no significant differences as the calculated t value was less than the tabulated one (4.30, n=3).

The precision of the developed method was evaluated by three concentrations of PGN within the linearity range. The obtained RSD% values for the within-day and between-days determination were within the range of 0.82-2.04% and 0.14–1.77%; n=3, at 390nm and 532nm respectively. These low values (less than or equal to 2%) reflected the precision of the developed method.

**Proposed reaction mechanism**

Ascorbic acid is oxidized by heat into dehydroascorbic acid (DHA). This step is essential for the reaction to take place (Pesez and Bartos, 1974).

As proposed in Scheme 1, the reaction proceeds by nucleophilic addition. The electron lone pair of the amino group in PGN attacks the most electrophilic carbonyl carbon in ascorbic acid to give an imine which undergoes hydrolysis into an amine. The amine then couples with another molecule of dehydroascorbic acid to give the coloured complex. This proposed mechanism is confirmed by the molar ratio results [drug: reagent] to be 1:2 (Figure 5).

**CONCLUSION**

The developed method was proven to be simple, accurate and precise for the determination of PGN in bulk and dosage forms. Ascorbic acid is considered a suitable, cheap and available reagent for the analysis of PGN. The developed method can be used for the routine analysis of PGN. Comparison of the developed method with the official methods is highly recommended to further prove the accuracy. Verification of the chemical structures of the formed complexes may contribute to better understanding of the reaction conditions. It is recommended to modify the developed methods in order to apply them to analyze PGN in biological samples.

**ACKNOWLEDGEMENT**

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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