Quantification of Aceclofenac and Pregabalin in Pharmaceutical Formulations using Nucleophilic Aromatic Substitution Reactions

Hitendra Kumar D. Gelani*, Payal P. Chauhan and Samir K. Shah

Department of Pharmaceutical Chemistry, Sardar Patel College of Pharmacy, Bakrol-Vadtal road, Anand, Gujarat, India.

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ABSTRACT

Aceclofenac and Pregabalin in combination have significant reduction in pain as compared to individual drug in chronic low back pain. Literature reveals that all the reported spectrophotometric methods are either need tedious extraction procedures do not offer high sensitivity, uses non specific reagent and/or recommend the measurement of absorbance in the near UV region where interference most probably occurs that do not offer suitable linearity range. A novel, accurate, precise and extraction free UV spectrophotometric method has been developed for simultaneous estimation of Aceclofenac and Pregabalin in combined dosage form. The method employed was Dual wavelength method. The method is based on the reaction of Pregabalin with 1,2-naphthaquinone-4-sulphonate sodium, yielding an orange colored product. The different experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized. Method involves measurement of Aceclofenac at 268.5 and 280.0 nm and measurement of Pregabalin at 324.0 and 339.5 nm. Rectilinear relationship with good regression coefficient 0.999 and 0.999 were found over the concentration ranges of 5-25 μg/ml and 3.75-18.75 μg/ml for Aceclofenac and Pregabalin, respectively with detection limit 0.62 and 0.50 μg/ml and Quantitation limit 1.87 and 1.52 μg/ml. The mean percentage recoveries were ranged 99.60 – 99.83 and 98.85 – 100.05 for Aceclofenac and Pregabalin, respectively. The developed methods were successfully applied to the analysis of the drug in its commercial tablet.

KEYWORDS: Aceclofenac; Pregabalin; 1,2-Naphthaquinone-4-sulphonate (NQS); Dual wavelength method.

Introduction

ACF is 2-[2-[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid. It is a non steroidal antiinflammatory drug with good analgesic (IP, 2010) Fig. 1. PGB is S-3-(amino methyl)-5-methylhexanoic acid. It is an anticonvulsant drug for neuropathic pain and adjunct for partial seizures. It can be used in generalised anxiety disorders. (Indian Pharmacopoeia, 2010) Fig. 2. ACF is official in British Pharmacopoeia-2009, Indian Pharmacopoeia-2010 and European Pharmacopoeia-2005(BP, 2010; IP, 2010; EP, 2011). PGB is official in Indian Pharmacopoeia-2010 (IP, 2010). The literature survey revealed that few analytical methods have been published concerning the simultaneous estimation of ACF and PGB either alone or in combination with other drug viz., spectrophotometric (Shah et al., 2008, Nikam et al., 2007) chromatographic (Shaikh et al., 2012, Jain et al., 2011 and Bharekar et al., 2011) methods for ACF and spectrophotometric (Oommen et al., 2013), chromatographic (Mishra et al., 2012) and also spectrofluorimetric (Onal et al., 2009 and Walash et al., 2011) methods for PGB. The chromatographic methods require high cost solvents in addition to elaborate treatment. On the other hand, Spectrofluorimeters are not available in many labs. Regarding spectrophotometric methods for determination of PGB, some of them do not offer high sensitivity or need tedious extraction procedures. Meanwhile, some of the spectrophotometric methods recommended the measurement of absorbance in the near UV region where interference most probably occurs or use non specific reagent (Potassium iodide/potassium iodate) that do not offer suitable linearity range. Therefore, our target was to develop rapid, simple, efficient and selective method for the analysis of ACF and PGB in pharmaceutical formulation.

Fig. 1. Structure of ACF.
To be UV sensitive, it is compulsory to have a chromophoric group in the structure. Thus, major aim of present research work is to introduce a chromophoric group in the structure and make it UV sensitive. This was achieved by converting primary amine group of PGB in UV sensitive product through reaction with chromogenic reagent. 1,2-Naphthoquinone-4-sulphonate sodium has been used as a chromogenic reagent for the analysis of many pharmaceutical primary and secondary amines. The proposed methods are based on the reaction of PGB through its primary amino group with 1,2-naphthaquinone-4-sulphonate (NQS) to form colored reaction product.

The main advantages of the proposed method are being novel, rapid and not require tedious extraction procedure. Compared to other reported spectrophotometric methods, the proposed method is either more sensitive or even having comparable sensitivity.

Materials and Methods

Reagents and Materials

ACF and PGB pure API were procured as a gratis sample from West Coast Pharmaceutical Works, Ahmedabad.

All employed chemicals were of analytical grade.

Instruments

A Systronics Double Beam UV-Visible Spectrophotometer 1 cm matched quartz cell was used for spectrophotometric measurements.

Swisser electronic balance was used for weighing the samples.

Preparation of standard stock solution

Accurately weighted quantity of ACF 100 mg and PGB 75 mg was transferred into two separate 100 ml volumetric flask, dissolved and diluted up to mark with Methanol to get strength of 1000 μg/ml of ACF and 750 μg/ml of PGB.

Preparation of working standard solution

Transfer 10 ml of stock solution of ACF and PGB into two separate 100 ml volumetric flask and diluted up to mark with Methanol to get strength of 100 μg/ml of ACF and 75 μg/ml of PGB.

Preparation of 1,2-Naphthaquinone-4-sulphonate (NQS) 0.5 % w/v

Accurately weighed 0.5 g of NQS was transferred into a 100 ml calibrated volumetric flask, dissolved in 5 ml distilled water and made up the volume with distilled water to obtain a solution of 0.5 % w/v. The solution was freshly prepared and protected from light during use.

Preparation of 0.01N Sodium hydroxide solution

Accurately weighed 0.2 g of sodium hydroxide and transferred into a 500 ml volumetric flask and made up to the mark with distilled water.

Calibration curve for ACF

The solution of Aceclofenac ranging from 5-25 μg/ml were prepared by pipetting out 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the working standard solution of Aceclofenac (100 μg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

The absorbance spectra of above solutions of Aceclofenac were recorded in the range of 200-400 nm. The difference in absorbance of 324.0 nm and 339.5.0 nm (difference is zero for Pregabalin) were plotted against the concentration of Aceclofenac to construct calibration curve for Aceclofenac.

Calibration curve for PGB

The solution of Pregabalin ranging from 3.75-18.75 μg/ml were prepared by pipetting out 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the working standard solution of Pregabalin (75 μg/ml) followed by adding 1 ml 0.5 % w/v NQS solution and 1 ml 0.01N NaOH into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

The absorbance spectra of above solutions of Pregabalin were recorded in the range of 200-400 nm. The difference in absorbance of 268.5 nm and 280.0 nm (difference is zero for Aceclofenac) were plotted against the concentration of Pregabalin to construct calibration curves for both the drugs.

Method Validation

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5-25 μg/ml and 3.75-18.75 μg/ml for ACF and PGB respectively.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 50%, 100% and 150% to the pre-analyzed sample. In this method the known concentration standard drug was added to the assay sample.

Precision

Precision of the method was determined by performing repeatability, intraday precision and interday
precision. In repeatability study, one concentration of both the drugs were analysed six time. In intraday precision, three replicates of three concentrations, were analyzed at short interval of time.In interday precision, three replicates of three concentrations, were analyzed at three consecutive day.

**LOD and LOQ**

The LOD and LOQ may be calculated as

LOD = \( 3.3 \times (SD / \text{Slope}) \)

LOQ = \( 10 \times (SD / \text{Slope}) \)

Where, \( SD \) = the standard deviation of \( Y \)-intercept of 5 calibration curves.

\( \text{Slope} = \) the mean slope of the 5 calibration curves.

**Assay of pharmaceutical formulation**

Twenty tablets were weighed and powdered. The tablet powder equivalent to 100 mg of ACF or 75 mg of PGB was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with Methanol to get strength of 1000 \( \mu \)g/ml ACF or 750 \( \mu \)g/ml PGB. The solution was filtered through Whatman filter paper no.42 and first few ml of filtrate were discarded. 10 ml of above solution (1000 \( \mu \)g/ml ACF or 750 \( \mu \)g/ml PGB) was transferred to 100 ml volumetric flask and volume was adjusted to the mark with Methanol to get strength of 100 \( \mu \)g/ml ACF and 75 \( \mu \)g/ml PGB. 1.0 ml of above solution (100 \( \mu \)g/ml ACF) was transferred to 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get strength of 10 \( \mu \)g/ml ACF. The absorbance of the solution was measured at 324.0 and 339.5 nm for determination of ACF. 1.0 ml of above remaining solution (75 \( \mu \)g/ml PGB) was transferred to 10 ml volumetric flask followed by adding 1 ml 0.5 % w/v NQS solution and 1 ml 0.01 N NaOH and volume was adjusted to the mark with Methanol to get strength of 7.5 \( \mu \)g/ml PGB. The absorbance of the solution was measured at 268.5 and 280.0 nm for determination of PGB. The concentration of each drug was calculated using equation of straight line.

**Results and Discussion**

**Optimization of derivatizing agent**

**Optimization of reagent concentration**

The influence of the reagent concentration was studied using different volume of 0.5% w/v solution of NQS. It was found that, increasing volume of the reagent produced a proportional increase in the absorbance value. Maximum absorbance was achieved using volume of the reagent ranged from 0.5-3.0 ml of NQS. Further increase of the reagent volume produced a gradual decrease in the absorption intensity. Therefore, 1 ml of 0.5% w/v NQS solution was chosen as the optimal volume of the reagent Fig. 3.

**Optimization of temperature**

The effect of the heating temperature on the formation of the reaction product was studied using a thermostatically controlled water bath at different temperature settings ranging from (30-80 °C). The result revealed that increasing the temperature resulted in an increase in the absorbance value of the reaction product. The maximum absorbance value was attained at 50 °C. At higher temperature, the absorbance of the reaction product decreased gradually. The decrease in the absorbance was probably attributed to the instability of the PGB derivative at higher temperature. Therefore, the study was carried out at 30 °C Fig. 4.

![Fig. 3. Effect of the volume of reagent on the absorbance of the reaction product of PGB with NQS.](image)

![Fig. 4. Effect of the temperature on the absorbance of the reaction product of PGB with NQS.](image)
NaOH in 10 ml volumetric flask up to mark with Methanol. Both solutions were scanned separately in the range of 200-400 nm. Data was obtained by overlay spectra of both drugs. From the overlay spectra two wavelengths 268.5 nm and 280.0 nm were selected as $\lambda_1$ and $\lambda_2$ for the estimation of Pregabalin where Aceclofenac shows the same absorbance at these wavelengths. Similarly, wavelengths 324.0 nm and 339.5 nm were selected as $\lambda_1$ and $\lambda_2$ for estimation of Aceclofenac where Pregabalin shows the same absorbance at these wavelengths.

### Method Development

Standard solution of Aceclofenac and Pregabalin were scanned separately in the range of 200-400 nm. Data was obtained by overlay spectra of both drugs. From the overlay spectra two wavelengths 268.5 nm and 280.0 nm were selected as $\lambda_1$ and $\lambda_2$ for the estimation of Pregabalin. Aceclofenac shows the same absorbance at these wavelengths. Similarly, wavelengths 324.0 nm and 339.5 nm were selected as $\lambda_1$ and $\lambda_2$ for estimation of Aceclofenac. Pregabalin shows the same absorbance at these wavelengths.

### Method Validation

#### Linearity and Range

The calibration curve for ACF and PGB was found to be linear in the concentration range 5-25 µg/ml and 3.75-18.75 µg/ml, respectively Fig. 7 and 8, Table-1.

#### Accuracy (Standard Addition Method)

Result obtained reveals that % recovery of ACF and PGB were found to be 99.60-99.83 and 98.85-100.05, respectively (Table 2).

#### Precision

For, Repeatability, % CV was found to be 1.23 and 0.84 for ACF and PGB, respectively. For, Intraday precision, % CV was found to be 0.40 – 1.12 and 0.61 – 0.94 for ACF and PGB, respectively. For, Interday precision, % CV was found to be 0.83 – 1.10 and 0.81 – 1.24 % for ACF and PGB, respectively.
TABLE 1
Linearity data for ACF and PGB.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Mean Absorbance Difference Between 324.00 nm and 339.5 nm ± SD (n=5)</th>
<th>% CV</th>
<th>Mean Absorbance Difference Between 268.5 nm and 280.00 nm ± SD (n=5)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.0271 ± 0.00015</td>
<td>0.55</td>
<td>0.0080 ± 0.00041</td>
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<tr>
<td>10</td>
<td>0.0322 ± 0.00017</td>
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<tr>
<td>15</td>
<td>0.0374 ± 0.00019</td>
<td>0.51</td>
<td>0.0165 ± 0.0013</td>
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<tr>
<td>20</td>
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<td>0.48</td>
<td>0.0207 ± 0.0017</td>
<td>0.82</td>
</tr>
<tr>
<td>25</td>
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<td>0.23</td>
<td>0.0247 ± 0.0014</td>
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</table>

TABLE 2
Accuracy (% Recovery study) (n=3).

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% of Std. Drug Added</th>
<th>% Recovery (Mean ± S.D)</th>
<th>% CV</th>
<th>Conc. (µg/ml)</th>
<th>% of Std. Drug Added</th>
<th>% Recovery (Mean ± S.D)</th>
<th>% CV</th>
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<tr>
<td>10</td>
<td>98.82 ± 1.70</td>
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<td>50</td>
<td>98.85 ± 0.46</td>
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<tr>
<td></td>
<td>100</td>
<td>99.60 ± 1.52</td>
<td>1.53</td>
<td>100</td>
<td>99.73 ± 0.40</td>
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<tr>
<td></td>
<td>150</td>
<td>99.83 ± 1.16</td>
<td>1.16</td>
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<td>100.05 ± 0.95</td>
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</table>

**LOD and LOQ**

LOD was found to be 0.62 and 0.50 µg/ml for ACF and PGB, respectively. LOQ was found to be 1.87 and 1.52 µg/ml for ACF and PGB, respectively.

**Assay of Marketed Formulation**

Percentage Purity of ACF and PGB were found to be 98.89% and 99.17% for ACF and PGB, respectively (Table 3).

**Conclusions**

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of ACF and PGB in combined dosage form. The method utilizes easily available and cheap solvent for analysis of ACF and PGB hence the method was also economic for estimation of ACF and PGB from combined dosage form. The common excipients and additives are usually present in the combined dosage form do not interfere in the analysis of ACF and PGB in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined dosage form.

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Address correspondence to: Hitendrakumar D. Gelani, Department of Pharmaceutical Chemistry, Sardar Patel College of Pharmacy, Bakrol Vadatal Road, Anand, Gujarat, India.

E-mail: hitendragelani13@gmail.com