Determination of Pregabalin in Bulk Drug and Pharmaceutical Formulations using Validated Stability-Indicating Spectrophotometric Methods

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Abstract- The present study describes the development and subsequent validation of stability-indicating, accurate, reliable, and sensitive spectro-photometric methods for the determination of Pregabalin in presence of its degradation products, including Dual wavelength and Ratio derivative after derivatization with vanillin reagent. With the Dual wavelength technique, Pregabalin could be determined in the range of 40-160 μg/mL at 390nm and 395.8nm. With the Ratio derivative technique, it could be determined in the above ranges at 401.6nm. All the methods were validated according to the International Conference on Harmonization guidelines and successfully applied to determine Pregabalin in pure form, laboratory-prepared mixtures, and pharmaceutical formulation. The obtained results were statistically compared with reported methods of analysis and there were no significant differences with respect to accuracy and precision of the adopted techniques.

Keywords: fibromyalgia; pregabalin; spectrophotometric methods; dual wavelength; ratio derivative; derivatization; vanillin reagent.

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Keywords: fibromyalgia; pregabalin; spectro-photometric methods; dual wavelength; ratio derivative; derivatization; vanillin reagent.

I. INTRODUCTION

Pregabalin (Figure 1) is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures. It has also been found effective for generalized anxiety disorder.

Recent studies have shown that pregabalin is effective at treating chronic pain in disorders such as fibromyalgia and spinal cord injury.

No spectrophotometric methods were reported in major pharmacopoeias like USP, EP and BP for determination of Pregabalin. Literature survey revealed many analytical methods for estimation of Pregabalin. However, no stability-indicating spectrophotometric method has been developed for bulk and pharmaceutical formulations.

The proposed methods were found to be easier than published HPLC methods for the determination of Pregabalin, for there is no need to use an internal standard, gradient elution, and time programming to adjust wavelengths. Moreover, the proposed methods are the first spectrophotometric methods for the determination of these drugs in presence of their degradation products. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, less expensive, and less time-consuming than other published LC methods.

a) Theoretical Background

i. Dual wavelength

This technique is used for binary mixtures for determination of one component without interference from the other. Two wavelengths are selected where the difference in absorbance of one component at these selected wavelengths is found to be zero, so, the difference in absorbance reflects only the concentration of one of the two components in the mixture.

ii. Ratio Derivative Spectrophotometric method

Salinas et al. proposed the spectrophotometric method termed ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Their method is based on the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of a standard solution of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentration of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths.

II. MATERIALS AND METHODS

a) Chemicals, Pharmaceutical Formulations and Reagents

(a) Pregabalin: Obtained from Optimus Drugs Ltd (Hyderabad, India).

(b) 75 and 150 mg capsules of Pregabalin (Irenypathic®): Produced by Amoun Pharmaceuticals Inc. (Cairo, Egypt).

(c) Methanol, hydrochloric acid, sodium hydroxide, potassium permanganate, sulphuric acid, sodium sulphite, disodium hydrogen phosphate, citric acid anhydrous, ethyl alcohol (96%) and vanillin.—Analytical reagent grade were purchased from Scharlau (Scharlab S.L, Sentmenat, Spain)
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(d) Double distilled water.: Prepared by using Millipore Milli-Q plus water purification system (Millipore Corp., Billerica, MA).

b) Equipments
(a) Double-beam UV-Vis spectrophotometer (Shimadzu 1650 PC) connected to a computer fitted with UVP personal spectroscopy software version 2.42 (Shimadzu) was used to process absorption and derivative spectra.: (Shimadzu Corp., Kyoto, Japan).
(b) Hotplate (WiseStir) with temperature controller.: Used to carry out degradation studies for all solutions (Daian Scientific Co. Ltd, Korea).
(c) pH-meter (Orion).: Equipped with combined glass electrode for pH adjustment (Thermo Scientific).
(d) Ultrasonic bath.: Elma (Danbury, CT).

c) Preparation of solutions
ICH guidelines Q1A_R2 (2.1.2) and Q2_R1(part II - 1.2) don't mention specified conditions or reagents for stress testing of drug substance. So, mild conditions (0.1N HCl or 0.1N NaOH) were used at first but didn't give complete degradation. So, drastic conditions were used to achieve complete degradation. KMnO₄ was used instead of hydrogen peroxide because it gave better results and the degradation reaction could be controlled or stopped on the contrary with hydrogen peroxide where the reaction could not be stopped.

1) Stock standard solution.: Stock standard solution of Pregabalin (5 mg/mL) was prepared by dissolving 500 mg of the drug in water, sonicated and completed to volume with the same solvent in a 100 mL volumetric flask. Then, 5 mL were further diluted to 50 mL with water. The required concentrations were prepared by serial dilutions.

2) Oxidative-induced forced degradation of Pregabalin (1000 µg/mL). : In a conical flask, 10 mL 0.1N KMnO₄ were added on 10 mL from stock standard solution (5mg/mL), the conical flask was covered with a funnel, heated on a hot plate - adjusted at 140 °C - for 60 min, cooled, then 0.5 mL of 4.5M H₂SO₄ and 1M sodium sulphite solution were added till discoloration, excess sulphuric acid was neutralized with 1M NaOH using a calibrated pH meter, and completed to 50 mL with water. Complete degradation was checked using HPLC.

Pregabalin is only sensitive to oxidative degradation. Acid, base, dry heat degradation and photo-degradation were tried but no significant change in the peak area appeared, indicating stability of Pregabalin to acid, alkaline, thermal and Photo-degradation.

3) Sample preparation.: Separately, the contents of 75 mg and 150 mg capsules were mixed, an amount equivalent to 500 mg of Pregabalin was accurately weighed, volume was completed to 100 mL with water then sonicated for 15 minutes and filtered. Then, 1 mL was further diluted to 50 mL with water.

4) Vanillin reagent.: Two grams of vanillin were weighed, volume was completed to 50 mL with ethyl alcohol (96%)

5) Buffer pH 7.5.: Prepared by mixing 35.5 mL of 0.2M disodium hydrogen phosphate anhydrous with 64.5 mL of 0.1M citric acid anhydrous, pH adjusted to 7.5 with 1M NaOH.

d) Procedures
The absorption spectra of the intact drug and its degradates are strongly overlapped, so application of the traditional spectral techniques failed to resolve this problem (Figure 2). On the other hand, this spectral overlapping was sufficient to demonstrate the resolving power of the proposed methods.

Pregabalin exhibits a very low UV absorption and as a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods. Pregabalin contains a primary aliphatic amino group, which is known to react with many color reagents as vanillin. Literature 34 shows that maximum absorbance intensities were achieved using 2 mL of buffer at pH 7.5. It was also found that 2 mL of Vanillin reagent was sufficient for production of maximum and reproducible color intensity. Time required for complete reaction at room temperature was 40 min. Heating leads to decrease in absorbance so reaction was done at room temperature.

This was further applied to perform the below mentioned methods under [(2.4.1) and (2.4.2)] to determine pregabalin in presence of its degradation products.

i. Dual wavelength (DWL)
Laboratory prepared mixtures of different concentrations of the intact drug and its degradation product were recorded against blank in the range from 390nm to 430nm for Pregabalin (Figure 3). Determine the absorbance at 390nm and 395.8nm.

The concentrations of Pregabalin in each mixture was determined by calculating the difference in absorbance measured at these wavelengths.

ii. Ratio Derivative Spectrophotometric method (RDer)
The absorption spectra recorded in the previously mentioned method (2.4.1) was divided by its divisor of oxidative induced degrade spectrum and the first derivative of the absorption spectra obtained was computed. Pregabalin concentrations were determined in each mixture from the absorbance at the amplitudes 401.6nm (Figure 4).

e) Method validation
1. Linearity.: Accurately measured aliquots of stock standard solution (2.3.1) were separately transferred
into a series of 25 mL volumetric flasks, to produce 40 to 160 µg/mL, on each flask, 2.5 mL of oxidative-induced degradate (2.3.2) were added to produce 100 µg/mL, 2 mL of Vanillin reagent (2.3.4), 2 mL of buffer pH 7.5 (2.3.5), flasks were left in dark at room temperature for 40 minutes and then completed to volume with water. Each of these solutions was measured in triplicate as mentioned under (2.4.1) and (2.4.2).

2. Accuracy.: Assay of drug in bulk powder.— The mentioned procedures under (2.4.1) and (2.4.2) were repeated by measuring 80, 100, 120 µg/mL Pregabalin standard solutions, prepared from stock standard solution (2.3.1), in triplicate after reaction with Vanillin reagent (2.3.4) in presence of buffer pH 7.5 (2.3.5) and the concentrations of Pregabalin were calculated by the corresponding regression equation.

3. Specificity.: In three separate flasks, accurately measured aliquots of standard stock solution (2.3.1) were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of oxidative-induced degradates (2.3.2) were added to produce 80, 100, 120 µg/mL each on a flask, and treated as mentioned under (2.5.1). Each of these solutions was measured in triplicate as mentioned under (2.4.1) and (2.4.2).

4. Precision.: Six replicates of same concentration (100 µg/mL) were checked for repeatability. The intraday and interday variation for the determination of Pregabalin was carried out at three different concentration levels of 80, 100, 120 µg/mL as mentioned under (2.4.1) and (2.4.2) after treatment as mentioned under (2.5.1).

5. Assay of pharmaceutical dosage forms.: For determination of Pregabalin in 75 mg or 150 mg capsules, from the sample solutions (2.3.3), aliquots were transferred to 25 mL volumetric flasks, treated as mentioned under (2.5.1) to produce 100 µg/mL and then measured in triplicate as mentioned under (2.4.1) and (2.4.2).

Further, standard addition technique was followed: In three separate flasks, accurately measured aliquots of a previously analyzed sample solution of 75 mg capsules (2.3.3) were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of standard stock solution (2.3.1) were added to produce 20, 40, 60 µg/mL each on a flask, treated as mentioned under (2.5.1) and then measured in triplicate as mentioned under (2.4.1) and (2.4.2).

The same steps were repeated with sample solution 150 mg capsules (2.3.3).

III. Results And Discussion

a) Method validation

Method validation was performed according to the ICH guidelines for the suggested spectrophotometric methods.

Linearity.: was evaluated by analyzing different concentrations of Pregabalin in the ranges of 40 to 160 µg/mL. The analysis was performed according to the experimental conditions previously mentioned in (2.4.1) and (2.4.2). Results are summarized in (Table 1).

Accuracy.: The accuracy of the results was checked by applying the proposed methods for the determination of % recoveries of 3 different concentrations of the drug in bulk powder. The concentrations were obtained from the corresponding regression equation, and the recoveries were calculated (Table 4).

Precision.: Precision of the obtained results of three concentrations of Pregabalin (80, 100, 120 µg/mL) were evaluated by three replicate determinations to estimate the intraday and interday variations. Then, the RSD% was calculated (Table 1).

LOD and LOQ.: Approaches based on the SD of the intercept and the slope were used for determining the LOD and LOQ, where

LOD = 3.3 x SD/slope and LOQ = 10 x SD/slope

These were determined experimentally for the proposed methods and are presented in Table 1.

Specificity.: The developed methods were found to be specific and selective. The intact drug can be detected without interference from its degradation products and formulation excipients. Recovery and relative standard deviations were calculated. Results are summarized in Table 2.

b) Analytical applications

The proposed methods were successfully applied to commercial preparations, and the standard addition technique was performed. The concentrations were calculated using the corresponding regression equation. Results are summarized in Table 3.

c) Statistical analysis

A statistical comparison of the results obtained by the proposed methods and the reported method for determination of Pregabalin was done. The significant difference between groups was tested by t-test as shown in Table 5. The test ascertained that there was no significant difference with respect to accuracy and precision between the proposed methods and the reported method.

IV. Conclusion

The paper describes simple, inexpensive, precise, accurate, and sensitive methods for determination of Pregabalin in bulk drug as well as in
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pharmaceutical dosage forms, also, can separate the drug from its degradation products, so, can be described as stability-indicating assay methods. The minimum sample preparation and the speed of analysis are the main advantages of these methods over other analytical procedures, unlike the HPLC procedures, the instrument is simple and inexpensive using a small quantity of reagents, thus, cost and time saving.

Acknowledgements
Authors are thankful to Amoun Pharmaceuticals for providing excellent facilities for carrying out this research work.

References Références Referencias

### Table 1: Calibration data for the determination of Pregabalin by the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Range μg/mL</th>
<th>Regression equation (Y = bC + a)</th>
<th>(r^2)</th>
<th>LOD, μg/mL</th>
<th>LOQ, μg/mL</th>
<th>Repeatability RSD%</th>
<th>Intraday RSD%</th>
<th>Interday RSD%</th>
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<tbody>
<tr>
<td>DWL</td>
<td>40 - 160</td>
<td>(Y = 0.00 C - 0.021)</td>
<td>0.9998</td>
<td>3.092</td>
<td>9.369</td>
<td>0.573</td>
<td>0.862</td>
<td>0.803</td>
</tr>
<tr>
<td>RDer</td>
<td>60 - 140</td>
<td>(Y = 0.012 C + 0.069)</td>
<td>0.9992</td>
<td>6.022</td>
<td>18.25</td>
<td>0.139</td>
<td>0.117</td>
<td>0.206</td>
</tr>
</tbody>
</table>

*aa* = Intercept, *b* = slope, and *C* = concentration of drug in μg/mL

\(P+OD=\) Pregabalin and oxidative degradates

\(DWL=\) Dual wavelength, \(RDiff=\) Ratio difference, \(RDer=\) Ratio Derivative

### Table 2: Specificity results of Pregabalin using the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Claimed conc. (μg/mL)</th>
<th>Imp. conc. added (μg/mL)</th>
<th>Recovery %</th>
<th>Av. recovery ±RSD%</th>
<th>Method</th>
<th>Recovery %</th>
<th>Av. recovery ±RSD%</th>
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<tbody>
<tr>
<td>DWL</td>
<td>100</td>
<td>80</td>
<td>100.96</td>
<td>100.32 ±0.735</td>
<td>RDer</td>
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<td>101.23 ±1.024</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>99.52</td>
<td></td>
<td></td>
<td>100.05</td>
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<tr>
<td></td>
<td></td>
<td>120</td>
<td>100.48</td>
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<td>101.98</td>
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### Table 3: Application of standard addition technique for the determination of Pregabalin in formulations using the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Claimed conc. (μg/mL)</th>
<th>Std conc. added (μg/mL)</th>
<th>Recovery % (dosage form 1)</th>
<th>Recovery % (dosage form 2)</th>
<th>Method</th>
<th>Recovery % (dosage form 1)</th>
<th>Recovery % (dosage form 2)</th>
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<tbody>
<tr>
<td>DWL</td>
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<td>20</td>
<td>100.00</td>
<td>100.00</td>
<td>RDer</td>
<td>98.14</td>
<td>98.14</td>
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<tr>
<td></td>
<td></td>
<td>40</td>
<td>98.84</td>
<td>100.00</td>
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<td>98.45</td>
<td>98.39</td>
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<td></td>
<td></td>
<td>60</td>
<td>101.55</td>
<td>99.22</td>
<td></td>
<td>98.18</td>
<td>98.43</td>
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<tr>
<td></td>
<td></td>
<td>Av. recovery ±RSD%</td>
<td>100.13 ±1.59</td>
<td>99.74 ±0.448</td>
<td></td>
<td>98.25 ±0.171</td>
<td>98.32 ±0.159</td>
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### Table 4: Results of recovery studies of Pregabalin

<table>
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<tr>
<th>Method</th>
<th>Theoretical conc. (μg/mL)</th>
<th>Actual conc found (μg/mL)</th>
<th>Recovery %</th>
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<tbody>
<tr>
<td>DWL</td>
<td>80</td>
<td>78.97</td>
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<td></td>
<td>100</td>
<td>97.28</td>
<td>97.28</td>
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<tr>
<td></td>
<td>120</td>
<td>100.76</td>
<td>120.91</td>
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<table>
<thead>
<tr>
<th>Method</th>
<th>Theoretical conc. (μg/mL)</th>
<th>Actual conc found (μg/mL)</th>
<th>Recovery %</th>
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<tbody>
<tr>
<td>RDer</td>
<td>80</td>
<td>79.85</td>
<td>99.81</td>
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<tr>
<td></td>
<td>100</td>
<td>100.37</td>
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<td></td>
<td>120</td>
<td>120.38</td>
<td>100.32</td>
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Table 5: Statistical comparison between the results of the proposed spectrophotometric methods and the reported method for determination of Pregabalin

<table>
<thead>
<tr>
<th>Statistical term</th>
<th>Reported method</th>
<th>DWL (P+OD)</th>
<th>RDer (P+OD)</th>
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</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>mean recovery</td>
<td>100.258</td>
<td>100.13</td>
<td>98.25</td>
</tr>
<tr>
<td>Variance</td>
<td>0</td>
<td>1.852</td>
<td>0.028</td>
</tr>
<tr>
<td>t-test</td>
<td>2.776</td>
<td>0.164</td>
<td>-20.58</td>
</tr>
<tr>
<td>(t-tabulated)</td>
<td></td>
<td>(t-calculated)</td>
<td>(t-calculated)</td>
</tr>
<tr>
<td><strong>Precision - intraday</strong></td>
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<td></td>
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<tr>
<td>mean recovery</td>
<td>100.059</td>
<td>100.36</td>
<td>99.25</td>
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<tr>
<td>Variance</td>
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<td>0.918</td>
<td>0.986</td>
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<td>0.117</td>
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<tr>
<td>t-test</td>
<td>2.776</td>
<td>0.487</td>
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<td><strong>Precision - interday</strong></td>
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<tr>
<td>mean recovery</td>
<td>100.02</td>
<td>100.14</td>
<td>99.09</td>
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<td>Variance</td>
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<td>RSD% mean</td>
<td>0.762</td>
<td>0.803</td>
<td>0.206</td>
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<tr>
<td>t-test</td>
<td>2.776</td>
<td>0.18</td>
<td>-1.56</td>
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Figure 1: Pregabalin [(S)-3-(aminomethyl)-5-methylhexanoic acid] intact drug

Figure 2: Pregabalin 0.1mg/ml (blue) + oxidative degradation 0.1mg/ml (red)

Figure 3: Pregabalin (40 - 160µg/ml) + oxidative degradation (100µg/ml) by Dual wavelength
Figure 4: Pregabalin (40 - 160µg/ml) + oxidative degradation (100µg/ml) by Ratio derivative