DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF PREDNISOLONE IN BULK AND TABLETS USING UV-VISIBLE SPECTROSCOPY

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ABSTRACT
Prednisolone is used as anti-inflammatory or immune suppressive agent. Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed two new, precise and simple UV spectrophotometric methods for estimation of Prednisolone from tablet formulation. The drug obeyed the Beer's law and showed good correlation. Absorption maxima of Prednisolone in methanol were found to be at 244 nm. Beer's law was obeyed in concentration range 2 – 12 mcg/ml. The absorbance was found to increase linearly with increasing concentration of prednisolone, which is corroborated by the calculated correlation coefficient value of 0.9995 (n=6). The results of analysis were validated by recovery studies. The recovery was more than 98%. The method was found to be simple, accurate, precise and economical.

Keywords: Prednisolone, UV Spectrophotometric, AUC, Linearity.

INTRODUCTION
Chemically Prednisolone is a glucocorticoid and its IUPAC name is (8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-17,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-3-one. Prednisolone is used as anti-inflammatory or immune suppressive agent and it is official in India Pharmacopoeia. In our Literature survey reveals that for Prednisolone HPLC and solid phase extraction methods have been reported for its determination in commercial formulation. However some of these methods are costlier and time consuming. To overcome these difficulties Spectrophotometric analysis serves to be the quickest, promising and reliable method for routine analytical needs. The aim of the present study is to develop two new simple, rapid, reliable and precise UV Spectrophotometric methods for analysis of Prednisolone from tablet formulation; first method is based on measurement of UV absorbance of Prednisolone at 244 nm in methanol. Second method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 241 nm and 247 nm. Area calculation processing item calculates the area bound by the curve.

MATERIALS AND METHODS
Chemicals
Gift samples of Prednisolone were provided by floors (India). Methanol (Qualigens laboratory, Mumbai). All solutions were prepared daily.

Instrumentation and analytical conditions
The UV method was performed on a Double-beam Shimadzu UV-Visible spectrophotometer, 1700, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution. Working wavelength for UV method was 244nm of Prednisolone.

Preparation of Standard Solutions
A stock solution containing 100mcg/ml of pure drug was prepared by dissolving accurately weighed 10mg of Prednisolone in methanol and volume was adjusted to 100ml with the same in 100ml volumetric flask.

Linearity and Calibration
The aliquots working standard solution was diluted serially with methanol to obtain the concentration range of 2 – 12 mcg/ml. A calibration curve for Prednisolone was obtained by measuring the absorbance at the Amax of 244 nm (for method I) and by measuring area under curve between 241 to 247 (for method II). Statistical parameters like the slope, intercept, coefficient of correlation, standard deviation, relative standard deviation, and standard error were determined.

Analysis of marketed tablet formulation
Accurately weighed the 20 tablets and powdered. The powder equivalent to 5mg of Prednisolone was transferred to 100ml volumetric flask and 20ml methanol is added to dissolve the Prednisolone in it and made the volume to mark with the same. This mixture was sonicated for 10 minutes and filtered through Whatmann filter paper No. 41. Aliquots (0.1 ml six times) of the sample were removed and diluted to 10 ml with methanol to obtain strengths as 10mcg/ml six time and determined the respective absorbance at 244 nm and area under curve between 241 nm to 247 nm against the methanol as blank. Recovery Studies
Recovery studies were performed to judge the accuracy of the method. 1ml of standard formulation (10mcg/ml) was taken in three 10ml volumetric flask and to it 80%, 100% and 120% (i.e. 0.8ml, 1.0ml, 1.2ml) of working standard solution (100mcg/ml) added respectively and made the volume up to the mark. The respective absorbance at 244 nm and area under curve between 241 nm to 247 nm was recorded against the blank. The amount of added concentration was determined from the obtained absorbance values and percent recovery was determined for the formulation.

RESULTS
The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 244 nm, shown in (fig.1). The Beer’s law was verified from the calibration curve by plotting graphs of concentration vs absorbance (method I) and concentration vs area under curve (method II). Regression analysis showed very good correlation. The calibration plots revealed zero intercept which is clear by the regression analysis equation Y = 0.0642X + C. Where Y is absorbance, m is the slope and X is the concentration of Prednisolone in mcg/ml as obtained by the least square method. The results thus obtained are depicted in (Table No.1). The results of analysis for assay and recovery studies for two different formulations were studied and are shown in (Table No.2). No significant variations were observed on interday and intraday analysis.
Table 1: Optical characteristics of Prednisolone

<table>
<thead>
<tr>
<th>Methods</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption maxima</td>
<td>244</td>
<td>241-247</td>
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<tr>
<td>Beer’s law limit</td>
<td>2-12mcg/ml</td>
<td>2-12mcg/ml</td>
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<tr>
<td>Coefficient of Correlation</td>
<td>0.9995</td>
<td>0.9990</td>
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<td>Regression equation</td>
<td>0.9969</td>
<td>0.9965</td>
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<td>Slope</td>
<td>0.0642</td>
<td>0.0631</td>
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<td>Y intercept</td>
<td>0.0352</td>
<td>0.0367</td>
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</table>

Table 2: Results of analysis of tablet and recovery study

<table>
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<tr>
<th>Method</th>
<th>Formulation</th>
<th>Label claim</th>
<th>%Label Claim</th>
<th>Standard Deviation</th>
<th>Coefficient of variation</th>
<th>%Recovery Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Wysolone</td>
<td>5mg</td>
<td>99.998</td>
<td>0.69043</td>
<td>0.00612</td>
<td>100.36±0.135</td>
</tr>
<tr>
<td>II</td>
<td>Wysolone</td>
<td>5mg</td>
<td>100.056</td>
<td>0.83055</td>
<td>0.00721</td>
<td>100.25±0.154</td>
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</tbody>
</table>

DISCUSSION

The spectrum of Prednisolone in methanol showed the absorption maxima at 244 nm. No effect of dilution was observed on the maxima, which confirmed the maxima at 244 nm. The statistical analysis of data obtained for the calibration curve of Prednisolone in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 2 – 12 mcg/ml (fig 2). The plot clearly showed a straight line passing through origin (y = 0.0642x + 0.0689). The estimated method was validated by low values of % RSD and standard error, indicating accuracy and precision of the methods. Excellent recovery studies further proves the accuracy of the method.
CONCLUSION

The two proposed methods based on Spectrophotometry were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. Hence, it can be concluded that the developed Spectrophotometric methods are accurate.

REFERENCES

3. Yoe-Ray Ku, Yi-Chu Liu And Jer-Huei Lin, Solid-phase Extraction and High-performance Liquid Chromatographic Analysis of Prednisone Adulterated in a Foreign Herbal Medicine, J of Food and Drug Anal. Vol. 5; No. 3; 2001; 150-152.