DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF METHYLPREDNISOLONE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The aim of present work was to develop an simple, accurate and precise zero order derivative method and Area Under Curve (AUC) method for the estimation of methylprednisolone and validation according to ICH Q2(R1) guideline. The absorbance maxima was determined at 242nm using methanol as a solvent. The area selected for the estimation of methylprednisolone was between 237 to 247nm. The method represented the the correlation coefficient (R²=0.999) at concentration range 5-25mcg/ml. the method were validated for the parameters such as linearity, accuracy, precision, and limit of quantification, limit of detection as per ICH guidelines. The proposed method were thus found suitable for estimation of methylprednisolone in bulk and tablet formulation without any interference of the excipients.

KEYWORDS: Methylprednisolone, AUC, Validation.

INTRODUCTION[1-2]

Methylprednisolone is a corticosteroid medication used to suppress the immune system and decrease inflammation, glucocorticoids can inhibit leukocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses. The anti-inflammatory actions of corticosteroids are thought to involve
phospholipase A₂ inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

![Structure of Methylprednisolone](image)

**Fig. 1: Structure of Methylprednisolone.**

On literature survey, it was found that only study of action of methylprednisolone in human plasma has been reported and no method is available in pharmacopeias. The proposed method were validated according to ICH guidelines.

**MATERIALS AND METHODS**

**INSTRUMENTS USED**
A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) connected to computer loaded with spectra manager software UV probe 4.21 with 10nm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400nm, scan speed: medium, sampling, interval: 1.0nm, band width (∆): 10.0nm, spectral slit width: 1nm. All weights were taken on electronic balance (model shimadzu AUX 120).

**Standard stock solution**
Methylprednisolone (100mg) was accurately weighed and transferred in a 100ml volumetric flask. Methanol was added to obtain a concentration of 1000mcg/ml (stock-1). From stock-1 10ml of solution was withdrawn and transferred to a 100ml volumetric flask and made up the volume with methanol to obtain a concentration of 10mcg/ml (stock-II). From the above stock solution-II aliquots of 0.5ml, 1ml, 1.5ml, 2ml,2.5ml were withdrawn and transferred into 10ml volumetric flasks and made up the volume with methanol to obtain a concentration of 5mcg/ml, 10mcg/ml, 15mcg/ml, 20mcg/ml, and 25mcg/ml respectively.

**Determination of maximum wavelength**
Methylprednisolone solution were scanned in UV spectrophotometer in the range of 200-400nm. Methanol was used as blank. Wavelength corresponding to maximum absorbance of methylprednisolone in methanol was observed at 242nm.
Preparation of standard calibration curve

Zero order derivative zero order derivative curve was obtained by measuring the absorbance of methanol solution in concentration range (5-25 mcg/ml) prepared from stock solutions in methanol at 242nm. Calibration curve of methylprednisolone was plotted with absorbance on y-axis and methylprednisolone concentration on x-axis.

![Graph showing absorbance vs concentration](image)

**Fig. 2: zero order derivative.**

Calibration curve methylprednisolone for AUC

This method involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelength $\lambda_1$ and $\lambda_2$. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated (237nm-347nm). This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrum were used to calculate AUC.

![Graph showing absorbance vs concentration](image)

**Fig. 3: plot of zero order derivative.**
VALIDATION\cite{4-8}

The developed method was validated as per ICH guidelines.

**Linearity**

The linearity was determined by using working standard solution between 5-25mcg/ml. The absorbance values of these solutions were measured at wavelength 242nm. The area under curve was integrated in wavelength range 237-247nm. Calibration curve of area under curve versus concentration was plotted after suitable calculation and simple linear regression was performed.

**Precision(reproducibility)**

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding response 3 times on the same day and 3 different days. The concentration of standard solution of methylprednisolone 15mcg/ml. area under curve of each of these
solution was measured in the range of 237-247nm. The result were reported in terms of relative standard deviation (RSD) was calculated.

### Table 1: Statistical Data of Precision by Zero order derivative method.

<table>
<thead>
<tr>
<th>Component</th>
<th>Precision</th>
<th>Mean*</th>
<th>Standard Deviation*</th>
<th>Co-efficient of Variation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPN</td>
<td>Intra-day</td>
<td>100</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Inter-day</td>
<td>100.04</td>
<td>0.106105</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Table 1.2. Statistical Data of Precision by Area under curve method.

<table>
<thead>
<tr>
<th>Component</th>
<th>Precision</th>
<th>Mean*</th>
<th>Standard Deviation*</th>
<th>Co-efficient of Variation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPN</td>
<td>Intra-day</td>
<td>99.04</td>
<td>0.441059</td>
<td>0.44533</td>
</tr>
<tr>
<td></td>
<td>Inter-day</td>
<td>99.4</td>
<td>0.535288</td>
<td>0.5385</td>
</tr>
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</table>

### Accuracy

The accuracy for the analytical procedure was determined at 80% 100% and 120% level of standard solution. Area under curve was measured in the range 237-347nm and result were expressed in terms % recoveries. Three determination at each level were performed and %RSD was calculated.

### Table 1.3. Statistical Data of Recovery studies by Zero order derivative method.

<table>
<thead>
<tr>
<th>Level of % recovery</th>
<th>Mean*</th>
<th>Standard Deviation*</th>
<th>Co-efficient of Variation*</th>
</tr>
</thead>
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<tr>
<td>80%</td>
<td>101</td>
<td>0.52915</td>
<td>0.523</td>
</tr>
<tr>
<td>100%</td>
<td>100.3</td>
<td>0.321455</td>
<td>0.320</td>
</tr>
<tr>
<td>120%</td>
<td>99.80</td>
<td>1.304812</td>
<td>1.307</td>
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### Table 1.4. Statistical Data of Recovery studies by Area under curve method.

<table>
<thead>
<tr>
<th>Level of % recovery</th>
<th>Mean*</th>
<th>Standard Deviation*</th>
<th>Co-efficient of Variation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>99.4</td>
<td>0.960902</td>
<td>0.9667</td>
</tr>
<tr>
<td>100%</td>
<td>100.1</td>
<td>0.600694</td>
<td>0.6000</td>
</tr>
<tr>
<td>120%</td>
<td>100.5</td>
<td>1.006645</td>
<td>1.0016</td>
</tr>
</tbody>
</table>

### DISCUSSION

The attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of methylprednisolone in tablet dosage form. The correlation coefficient equation was y=0.0091x+0.0017 (R^2 =0.999). the area under curve between 237-247nm. x is concentration and R is correlation coefficient. The R^2 value as 0.999 as indicates that developed method was linear. The proposed method was found to be precise %RSD value for inter day and intraday precision were satisfactory. The drug at each of the good recoveries (100%). Hence, it can be said that this method was accurate. The result of the analysis of
pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable.

**CONCLUSION**

The developed UV spectroscopic area under curve method provide simple, precision and accurate, quantitative analysis for estimation of methylprednisolone in pharmaceutical dosage form. No any spectrophotometric methods have been described for AUC estimation of methylprednisolone. Therefore, simple, fast and reliable area under curve spectrophotometric method was developed for the routine analysis of methylprednisolone.

**REFERENCE**