DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DEXAMETHASONE SODIUM PHOSPHATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple efficient, precise and accurate spectroscopic method has been developed and validated for quantitative estimation of Dexamethasone sodium phosphate in bulk and pharmaceutical dosage form. Dexamethasone sodium phosphate is soluble in distilled water, so it was used as solvent. Dexamethasone sodium phosphate is dissolved in distilled water the resulting solution was then scanned in the UV range (200-400nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The λmax of Dexamethasone sodium phosphate was found to be 242nm. The method obeys Beers law in the concentration range from 5-25 μg/ml. The correlation coefficient was found to be 0.999 (r² 0.999). The LOD and LOQ were found to be 0.78 and 2.3μg/ ml respectively. The result of estimation of marketed formulation (Demisone) was found to be 94.19%. The accuracy of the method was determined by recovery studies. The percentage recovery was found to be 93.3%. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2. So, the proposed method was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for routine analysis of Dexamethasone sodium phosphate in bulk drug and the Pharmaceutical formulations.

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INTRODUCTION
Dexamethasone sodium phosphate is a potent anti-inflammatory and immunosuppressant drug, used to treat a number of inflammatory, allergic, rheumatic, endocrine, dermatologic, and autoimmune disorders. Dexamethasone sodium phosphate is also used in oncology patients, including those undergoing chemotherapy. It occurs as a white to creamy white powder, is exceedingly hygroscopic, is soluble in water and its solutions have a pH between 7.0 and 8.5. It has the following structural formula (figure 1)

![Figure 1: Dexamethasone sodium phosphate](image)

**Molecular Weight:** 516.4

**Molecular Formula:** C_{22}H_{28}FO_{8}PNa_{2}

**CAS Number:** 2392-39-4

**Synonym:** (11b, 16a)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione 21-phosphate disodium salt; Dexamethasone 21-Phosphate Disodium Salt; Ak-Dex; Baldex; Dalalone; Dezone; Hexadrol; Oradexon; Soldesam

PHARMACODYNAMICS:
Dexamethasone and its derivatives, dexamethasone sodium phosphate and dexamethasone acetate, are synthetic glucocorticoids. Used for its anti-inflammatory or immunosuppressive properties and ability to penetrate the CNS, dexamethasone is used alone to manage cerebral edema and with tobramycin to treat corticosteroid-responsive inflammatory ocular conditions.

MECHANISM OF ACTION:
Dexamethasone is a glucocorticoid agonist. Unbound dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic glucocorticoid receptors. This complex binds to DNA elements (glucocorticoid response elements) which results in a modification of transcription and, hence, protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of dexamethasone are thought to involve phospholipase A_{2} inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.
Literature survey reveals only one validated UV method was found for its quantitative determination in bulk and pharmaceutical dosage forms and in that method methanol : water was used as an solvent (1:2) ratio and in almost all cases simultaneous estimation was done for this drug by using antibiotics like Moxifloxacin, Literature survey reveals HPLC, RP-HPLC, with other drugs in combination.

**Objective:**
The objective of this work was to develop sensitive and efficient analytical methods for quantitative determination of dexamethasone sodium phosphate in bulk and pharmaceutical dosage forms. In this study, efforts were made to develop a simple, easy, and economic UV spectrophotometric method using distilled water as solvent for the determination of dexamethasone sodium phosphate in the bulk drug and dosage form. The developed method was optimized validated as per the guidelines of International Conference on Harmonization and demonstrated excellent linearity, precision, accuracy for Dexamethasone sodium phosphate.

**MATERIALS AND METHODS:**
**Instruments:** UV-Visible Double Beam Spectrophotometer (Thermo Scientific, Evolution 201) with 1cm matched Quartz cells, micro pipette of variable volume and Digital Balance.

**Chemicals:**
**Standard Solution of Dexamethasone sodium phosphate:**
Standard Dexamethasone sodium phosphate of 10mg was accurately weighed and transferred to 10ml volumetric flask. It was dissolved properly and diluted to mark with distilled water to obtain concentration of 1mg/ml. This solution was used as stock solution. From this, working standard solution and suitable dilutions were prepared.

**Determination of Absorption Maxima:**
By the appropriate dilution of standard drug solution with distilled water, solution contain 10µg/ml of Dexamethasone sodium phosphate was scanned in the range of 200-400nm to determine the wavelength of maximum Absorption. Drug showed Absorption maxima at 242nm. (Fig-2)

![Absorption maxima of dexamethasone sodium phosphate](image)

*Figure 2: Absorption maxima of dexamethasone sodium phosphate*
Procedure:
Aliquots of standard solution of Dexamethasone sodium phosphate ranging from 0.5-2.5 (1ml=100µg) were transferred in to a series of 10ml volumetric flasks. To each flask required amount of distilled water was added and finally the volume in each flask was brought up to the 10ml with the distilled water. Then Absorbance’s were measured at 242nm against the reagent blank. Then Calibration data was indicated in (Table-1)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.201</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.350</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.485</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.628</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0.755</td>
</tr>
<tr>
<td>6</td>
<td>10(test)</td>
<td>0.329</td>
</tr>
</tbody>
</table>

**Table-1:** Calibration Data of Dexamethasone sodium phosphate

calibration curve was plotted by taking concentration on X-axis and Absorbance on Y-axis which is shown in (Fig-3).

**Figure 3:** Calibration Curve of Dexamethasone sodium phosphate

Analysis of pharmaceutical dosage form (Demisone):
20 tablets were weighed accurately and were finely powdered. Tablet powder equivalent to 5mg of Dexamethasone sodium phosphate was transferred to a 50ml volumetric flask and 20ml of distilled water was added. The flask was sonicated for 10 minutes to solubilize the drug and the volume was made up to 50 ml using distilled water (100µg/ml). After filtration, 1ml of filtrate was transferred to 10ml volumetric flask and it was diluted to mark with distilled water and the absorbance of this solution was noted at 242nm against corresponding blank. (Table-2)

**Table 2:** Results obtained in the determination of Dexamethasone sodium phosphate in tablets.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount declared(mg/tab)</th>
<th>Amount Found (mg/tab)</th>
<th>Amount Found (10mg)</th>
<th>Confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone sodium phosphate</td>
<td>0.5mg</td>
<td>0.470mg</td>
<td>9.419mg</td>
<td>94.19%</td>
</tr>
</tbody>
</table>
VALIDATION PARAMETERS OF METHOD

Linearity:
An aliquot of concentration of 5-40µg/ml were prepared in duplicate, but linearity was found to be between 5-25µg/ml concentrations. The linearity was calculated by the Least Square Regression Method.

Precision:
The precision of the assay was determined by repeatability and reported as RSD%. For this 20µg/ml concentration was taken and measured 6 times in day and same was measured in the next day. The RSD% was calculated.

Table 3: Precision - 20µg/ml concentration was taken

<table>
<thead>
<tr>
<th>S.No</th>
<th>Intra-day Precision</th>
<th>%RSD</th>
<th>Inter-day precision</th>
<th>%RSD</th>
<th>Mean % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.628</td>
<td></td>
<td>0.608</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.632</td>
<td></td>
<td>0.614</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.619</td>
<td>0.748%</td>
<td>0.603</td>
<td>0.854%</td>
<td>0.801%</td>
</tr>
<tr>
<td>4</td>
<td>0.631</td>
<td></td>
<td>0.619</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.625</td>
<td></td>
<td>0.609</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.627</td>
<td></td>
<td>0.609</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accuracy:
The accuracy of the method was evaluated through Standard Addition Method. In this, known amount of standard Dexamethasone sodium phosphate was added in pre-analyzed sample. This was done for 10µg/ml concentration taking it as 100% for 3 times. It was done for 80%, 100%, 120% and the Recovery studies were performed and finally the RSD% was calculated.

Table 4: Accuracy - 10µg/ml concentration was taken as 100%

<table>
<thead>
<tr>
<th>Conc. Total Mean (µg/ml)</th>
<th>Spike level</th>
<th>Mean Absorbance</th>
<th>Mean Amount found</th>
<th>% Recovery</th>
<th>Mean % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>80%</td>
<td>0.564</td>
<td>17.89</td>
<td>98.99%</td>
<td>1.82%</td>
</tr>
<tr>
<td>10</td>
<td>100%</td>
<td>0.596</td>
<td>19.05</td>
<td>90.5%</td>
<td>1.24%</td>
</tr>
<tr>
<td>10</td>
<td>120%</td>
<td>0.651</td>
<td>21.06</td>
<td>90.6%</td>
<td>1.214%</td>
</tr>
</tbody>
</table>

Robustness:
It is a study of small but deliberate variations in method parameters such as Absorption Maxima, P^H and ratio of mobile phase solvents. In this present work the Absorption maxima was decreased and increased 2nm and carried the process for 20µg/ml solution for 6 times. The RSD% was calculated.

Table 5: Robustness-20µg/ml concentration was taken

<table>
<thead>
<tr>
<th>S.No</th>
<th>At 240nm (-2nm) %RSD</th>
<th>At 244nm (+2nm) %RSD</th>
<th>Mean % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.630</td>
<td>0.617</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.623</td>
<td>0.609</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.631</td>
<td>0.619</td>
<td>1.3%</td>
</tr>
<tr>
<td>4</td>
<td>0.629</td>
<td>0.603</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.627</td>
<td>0.601</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.628</td>
<td>0.602</td>
<td></td>
</tr>
</tbody>
</table>
Ruggedness:
It is the degree of reproducibility of test results obtained by the variety of conditions like different analysts, reagents, laboratories, days, equipment etc. The present work was performed by the change of analyst. Then nearly same results were obtained which are similar to that of first analyst.

Limit of detection (LOD):
It is the smallest quantity of an analyte that can be detected, and not necessarily determined, in a quantitative fashion. It was calculated by the following formula;

\[
LOD = 3.3 \times \frac{S.D}{slope}
\]

Where; S.D=Standard Deviation

Limit of Quantization (LOQ):
It is the lowest concentration of an analyte in a sample that may be determined with acceptable accuracy and precision. It was calculated by the following formula;

\[
LOQ = 10 \times \frac{S.D}{slope}
\]

Where; S.D=Standard Deviation

Table 6: Validation Parameters

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absorption (nm)</td>
<td>242nm</td>
</tr>
<tr>
<td>2</td>
<td>Linearity range (µg/ml)</td>
<td>5-25(µg/ml)</td>
</tr>
<tr>
<td>3</td>
<td>Standard Regression Equation</td>
<td>(Y=0.027x+0.068)</td>
</tr>
<tr>
<td>4</td>
<td>Correlation Coefficient ((r^2))</td>
<td>0.999</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy (%RSD)</td>
<td>1.424%</td>
</tr>
<tr>
<td>6</td>
<td>Precision (%RSD)</td>
<td>0.801%</td>
</tr>
<tr>
<td>7</td>
<td>Robustness (%RSD)</td>
<td>0.875%</td>
</tr>
<tr>
<td>8</td>
<td>Detection Limit</td>
<td>0.78 (µg/ml)</td>
</tr>
<tr>
<td>9</td>
<td>Quantization Limit</td>
<td>2.30(µg/ml)</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
A simple, selective, accurate, precise spectroscopic method for the estimation of Dexamethasone sodium phosphate in bulk and pharmaceutical dosage form has been developed and validated. The linearity range in the concentration range of 5-25µg/ ml \((r^2= 0.999)\). It indicated that the concentrations of Dexamethasone sodium phosphate had good linearity. The LOD and LOQ were found to be 0.78 and 2.3µg/ ml respectively. The amount of Dexamethasone sodium phosphate was calculated as 94.19%. Further the precision of the method was confirmed by the repeatable analysis of solution. The % RSD was found to be 0.801%. It indicated that the method has good precision. The percentage recovery was found to be in the range of 90.5-98.9%. The procedure was repeated for 3 times by taking 10µg/ml as 100%. The recovery was calculated for 80%, 100% and 120%. The % RSD was found to be 1.424%. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed.

CONCLUSION
The proposed method is simple, accurate, precise and selective for the estimation of Dexamethasone sodium phosphate in bulk and pharmaceutical dosage forms. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. Hence it can be effectively applied for the routine analysis of Dexamethasone sodium phosphate in bulk and marketed formulation.

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