ANALYSIS OF BETAMETHASONE DISODIUM PHOSPHATE INJECTION AND OPHTHALMIC SOLUTION BY HPLC, KINETIC INTERPRETATION AND DETERMINATION OF SHELF LIFE

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Abstract
Corticosteroids are widely used in therapeutics in different formulations. Betamethasone disodium phosphate is one of the most soluble of the adrenocorticosteroidal agents. It is therefore very suitable for intravenous use and particularly for ophthalmic formulations.

Acceleration method is used to determine expiration date of aqueous formulations manufactured in Iran.

INTRODUCTION
In the United States Pharmacopeia (USP)¹, monographs for cream, injectable, ophthalmic
ointment, ophthalmic solution and tablet formulations are found. The analytical procedures for the assay of ophthalmic and injectable preparations use no internal standard. On the other hand, experience has shown that use of an internal standard gives better results in terms of accuracy and precision of assay. Therefore, there was a need to improve and develop an analytical procedure for these formulations.

Previously, certain studies about determination of different pharmaceutical dosage forms of corticosteroids were accomplished. The use of accelerated stability studies at high temperature using the Arrhenius equations is a routine procedure for estimating the stability of aqueous drug at room temperature.

The method of accelerated testing of pharmaceutical products based on the principles of chemical kinetic was demonstrated by Garrett and Carper. According to this technique, the k values for the decomposition of a drug in solution at various elevated temperatures are obtained by plotting some functions of concentration against time.

The logarithms of the specific rates of decomposition are then plotted against the reciprocals of the absolute temperatures, and the resulting line is extrapolated to room temperature. The k_{25} is used to obtain a measure of the stability of the drug under ordinary shelf conditions.

**EXPERIMENTAL**

**Apparatus:**

The high performance liquid chromatograph consisted of a Perkin-Elmer LC Terminal, a Perkin-Elmer model LC4 flow pump, a 7125-075 Rheodyne injector, a LC-85B spectrophotometric detector and a Silica X_{10} (30cm x 4mm).

**HPLC OPERATING CONDITION:**

The mobile phase was aqueous-methanol (50-50) solution of 0.01M KH_{2}PO_{4}. The flow rate was 1 ml/min. The UV detector was operated at 254 nm and the sensitivity was 800-40. 20μl of either the standard or sample solution was injected into the column.
**INTERNAL STANDARD SOLUTION:**

A stock solution was prepared by weighing accurately about 100mg butylparaben, transferred into a 100-ml volumetric flask, dissolved in methanol and diluted to the volume with methanol.

**STANDARD SOLUTION:**

a) A stock solution was prepared by weighing accurately about 100mg betamethasone disodium phosphate, transferred into a 25-ml volumetric flask, dissolved in distilled water and diluted to the volume with distilled water.

b) 3 ml aliquot of the stock solution of betamethasone disodium phosphate and 5 ml aliquot of internal standard solution were transferred to a 50-ml volumetric flask and diluted to the volume with the mobile phase.

**SAMPLE SOLUTION OF INJECTABLE FORMULATION:**

2 ml aliquot of injectable solution was transferred to a 25 ml volumetric flask and diluted to the volume with the mobile phase.

**SAMPLE SOLUTION OF OPHTHALMIC DROP:**

All the samples that were used belonged to one batch and were kept in a water bath in their factory containers.

The temperature was controlled by a precision thermostat. Seven samples were kept at a certain temperature (70, 80, and 90); at the convenient intervals, samples were removed and analysed, after cooling, by HPLC.

Rate constants and other kinetic parameters were calculated using Computer Programmed Square Regression Analysis.

**RESULTS**

Results obtained from the analysis of betamethasone disodium phosphate showed that the rate of decomposition of this compound is pseudo-first order, and "LogC" versus "t" yields a straight line. Table 1 shows k values obtained for betamethasone disodium phosphate at 70, 80,
and 90°C.

These results indicate that the use of the Arrhenius equation (Log k = Log A - Ea/2.303RT) at high temperatures could be used for the estimation of the shelf-life of the drug at room temperature. The logarithms of the specific rates of decomposition are plotted against the reciprocals of the absolute temperatures as shown in Figures 1 and 2, and the resulting line is extrapolated to room temperature. The k25°C is used to obtain a measure of the stability of the drug under ordinary shelf condition.

The result of this study suggest that, by considering the standard error and its effects on calculating the rate constants and expiration date, the room temperature shelf life of both drop and injectable solution are almost 400 days.

Table 1.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>k x 10^3 (day)^{-1}</th>
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<tbody>
<tr>
<td></td>
<td>Injectable Formulation</td>
</tr>
<tr>
<td>70</td>
<td>16.10 ± 1.39</td>
</tr>
<tr>
<td>80</td>
<td>34.70 ± 2.30</td>
</tr>
<tr>
<td>90</td>
<td>69.90 ± 6.24</td>
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Fig. 1.
Arrhenius plot for predicting stability of injectable formulation at room temperature.
A slope of $-3.98 \pm 0.043$, an intercept of $9.82 \pm 0.121$, a correlation coefficient of $-0.999$, $k_{25^\circ}$ of $0.000267 \pm 0.000003$ (day)$^{-1}$, and $t_{90\% \; 25^\circ}$ of $392.6 \pm 4.24$ days. Activation energy calculated is $18.212 \pm 0.2$ Kcal/mol.
Fig. 2.

Arrhenius plot for predicting stability of ophthalmic drop at room temperature.

A slope of $-4.15 \pm 0.58$, an intercept of $10.33 \pm 1.65$, a correlation coefficient of $0.99$, $k_{25^\circ}$ of $0.000238 \pm 0.00003$ (day)$^{-1}$, and $i_{90\% 25^\circ}$ of $440 \pm 61$ (days). Activation energy calculated is $18.99 \pm 2.6$ Kcal/mol.
REFERENCES

3) Li Wan, Po, A.; Irwin, W.J.; Yip, Y.W.; *J. Chromatogr.* 1979 Sep 1, 176 (3), 399-405.