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ANALYSIS OF DEXAMETHASONE SODIUM PHOSPHATE INJECTION AND OPHTHALMIC SOLUTION BY HPLC, KINETIC INTERPRETATION AND DETERMINATION OF SHELF LIFE

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Abstract

The use of accelerated temperature stability studies using the Arrhenius relationship is routine for estimating the stability of a drug at room temperature. Therefore, the acceleration method is used to determine the expiration date of dexamethasone aqueous formulation manufactured in Iran. A high-performance liquid chromatography (HPLC) method utilizing ultraviolet (UV) detection for dexamethasone disodium phosphate and ophthalmic solution is described. Using the kinetic information generated from the high-temperature studies, at 60, 70, 80 and 90°C, an Arrhenius plot for dexamethasone disodium phosphate degradation was constructed and the shelf-life of the drug was estimated.

Introduction

Corticosteroids are widely used in therapeutics in different formulations. Dexamethasone disodium phosphate has the same action as dexamethasone and is one of the most soluble of the adrenocorticosteroidal agents. It is therefore very suitable for intravenous use and particularly for ophthalmic formulations.

In the United States Pharmacopeia (USP) [1], monographs for creams, injectable and ophthalmic solutions, ophthalmic ointments and tablet formulations are found. The analytical procedures for the assay of injectable and ophthalmic preparations are HPLC but 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 on assay. Therefore, there was a need to improve and develop an analytical procedure for these formulations.

Certain studies on the stability of different pharmaceutical dosage forms and the mechanisms of this drug's decomposition have been previously carried out [2-5]. The use of accelerated stability studies at high temperatures using the Arrhenius equation is a routine procedure for estimating the stability of a drug at room temperature (25°C).

Keywords: Analysis; Dexamethasone; Stability

*Dr. Alireza Ghanbarpour, a distinguished professor at the College of Pharmacy, Medical Sciences University of Tehran, died on Oct. 9, 1993.

Experimental Section

A: Injectable Solution

Reagents

Dexamethasone disodium phosphate, methylparaben, propylparaben, creatinine and dexamethasone were USP grade. Acetophenone and KH_2PO_4 were reagent grade. Acetonitrile and water were distilled

HPLC grade.

Apparatus

The high-pressure liquid chromatograph consisted of a Waters model 510 flow pump, a Waters model UK6032935 manual injector, a Waters model 481 UV detector and a Millipore model 746 integrator recorder. The column was a μ bondapak C_{18} (Waters 300 × 3.9 mm).

HPLC Operating Condition

The mobile phase was aqueous acetonitrile (67.5: 32.5) solution of 0.01M KH_2PO_4. The mobile phase was degassed and filtered through a 0.45 μm filter (Millipore, type HVLP). The flow rate was 1 ml/min. The UV detector was operated at 254 nm and the sensitivity was 0.1 A.U.F.S. Instrument conditions were maintained for 1 h prior to the initial injection. 10 μl of either the standard or sample solution was injected into the column. After use, the column was prepared for storage by flushing with methanol.

Internal Standard Solution

0.15 ml aliquot of acetophenone was transferred into a 100-ml volumetric flask, dissolved in mobile phase, and diluted to the volume with mobile phase.

Standard Solution

a) A stock solution was prepared by weighing accurately about 100 mg of dexamethasone disodium phosphate, transferred to a 50-ml volumetric flask, dissolved in distilled water and diluted to the volume with distilled water.

b) 1 ml aliquot of the stock solution of dexamethasone disodium phosphate and 0.5 ml aliquot of internal standard solution were transferred to a 100-ml volumetric flask and diluted to the volume with distilled water.

Sample Solution

0.5 ml aliquot of injectable formulation and 0.5 ml aliquot of internal standard solution were transferred to a 100-ml volumetric flask and diluted to the volume with distilled water.

Calibration Curves

Six different calibration solutions were prepared as follows: 0.004, 0.008, 0.010, 0.020, 0.030 and 0.040 mg/ml of dexamethasone disodium phosphate with 0.5 ml of internal standard solution added. Calibration curves were obtained by plotting the peak area ratios of dexamethasone disodium phosphate and internal standard vs the respective calibration concentrations (Fig. 1).

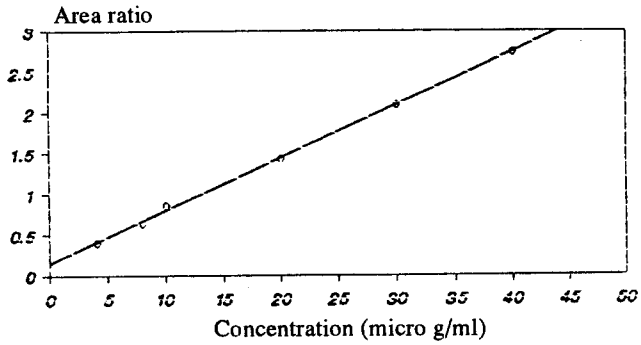

Figure 1. Calibration curves of dexamethasone disodium phosphate with six data points. A slope of 0.0656 ± 0.0011, an intercept of 0.1380 ± 0.0265 and a correlation coefficient of 0.9993 were calculated from the linear regression analysis.

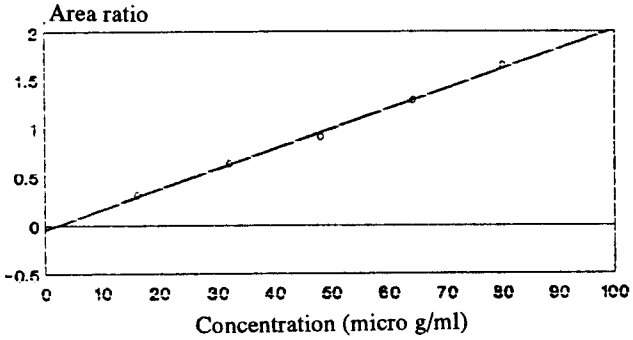

Figure 2. Calibration curves of dexamethasone disodium phosphate, with five data points. A slope of 20.7690 ± 0.0630, an intercept of - 0.051 ± 0.0330 and a correlation coefficient of 0.9986 were calculated from the linear regression analysis.

B: Ophthalmic Solution

Reagent

Dexamethasone disodium phosphate, betamethasone acetate and creatinine were USP grade. Methanol and water were distilled HPLC grade, KH_2PO_4 were reagent grade.

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 Sigma 15 chromatography data station.

HPLC Operating Condition

The mobile phase was aqueous methanol (50-50) solution of 0.01M KH_2PO_4. The flow rate was 1 ml/min. The UV detector was operated at 254 nm and the sensitivity was 800-40. 10 µl of either the standard or sample solution was injected into the column.

Internal Standard Solution

A stock solution was prepared by weighing accurately about 20 mg of betamethasone acetate, transferred into a 100-ml volumetric flask, dissolved in the mobile phase and diluted to the volume with the mobile phase.

Standard Solution

a) A stock solution was prepared by weighing accurately about 100 mg of dexamethasone disodium phosphate, transferred into a 50-ml volumetric flask, dissolved in distilled water and diluted to the volume with distilled water.

b) 2 ml aliquot of the stock solution of dexamethasone disodium phosphate and 10 ml aliquot of internal standard solution were transferred to a 50-ml volumetric flask and diluted to the volume with distilled water.

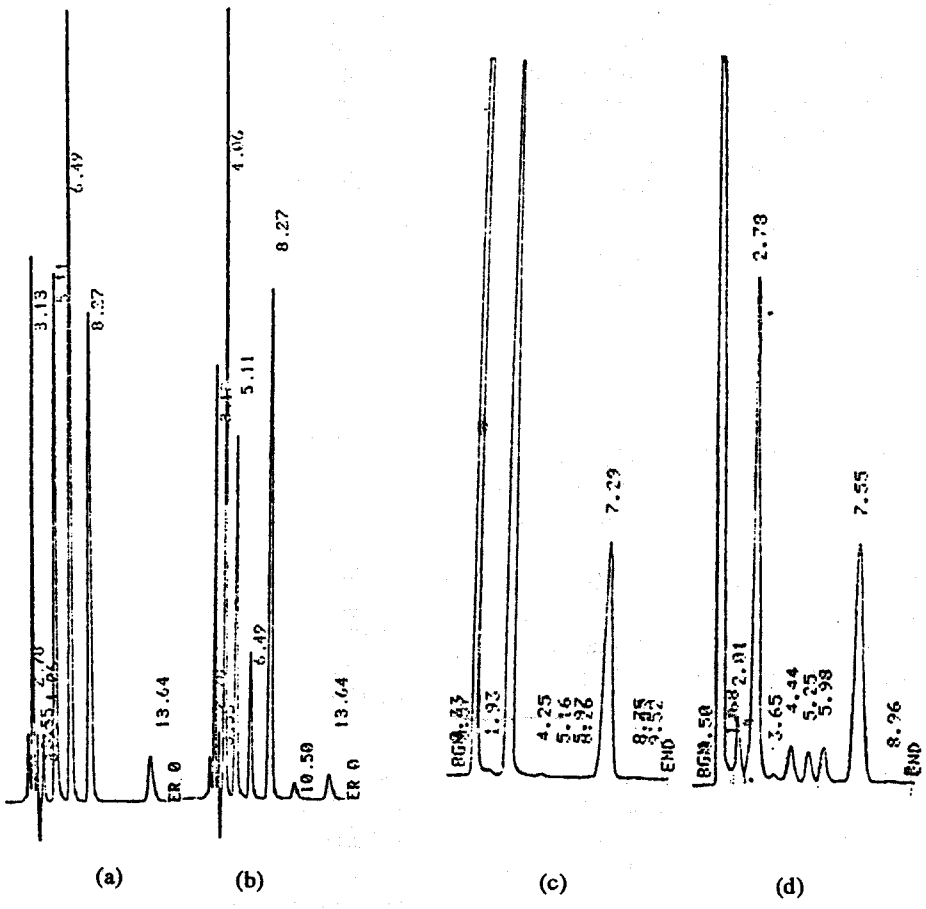

Figure 3. a) Chromatogram of original injectable formulation. b) Chromatogram of injectable formulation after its degradation at a high temperature (70°C, 2 weeks). Creatinine (3.13), dexamethasone phosphate (5.11), methylparaben (6.49), acetophenone (8.27), propylparaben (13.64), parahydroxy benzoic acid (4.06) and free dexamethasone (10.50). c) Chromatogram of original ophthalmic formulation. d) Chromatogram of ophthalmic formulation after its degradation at a high temperature (70°C, 17 days). Creatinine (1.17), dexamethasone phosphate (2.17), betamethasone acetate (7.29) and free dexamethasone (5.25). The other peaks (2.01, 3.65, 4.44 and 5.98) are related to plastic container of ophthalmic drop.

Sample Solution

2 ml aliquot of ophthalmic formulation and 5 ml aliquot of internal standard solution were transferred to a 25-ml volumetric flask and diluted to the volume with distilled water.

Calibration Curves

Five different calibration solutions were prepared as follows: 0.016, 0.032, 0.048, 0.640 and 0.080 mg/ml of dexamethasone disodium phosphate with 0.04 mg/ml of betamethasone acetate added. Calibration curves were obtained by plotting the peak area ratios of dexamethasone disodium phosphate and internal standard vs the respective calibration concentrations (Fig. 2).

Kinetic Measurements

All the samples 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 (60, 70, 80 and 90°C); at convenient intervals, samples were removed and analyzed, after cooling, by HPLC. Rate constants and other kinetic parameters were calculated using Computer Programmed Least Square Regression Analysis.

Results and Discussion

Figure 3 clearly indicates that it is possible to separate active ingredients, degradation products, excipients and other materials of interest.

Results obtained from the analysis of dexamethasone disodium phosphate show that the rate of decomposition of this compound is pseudo first order, and "LogC" versus "t" yields a straight line. Table 1 shows the results of the analysis of dexamethasone disodium phosphate.

These results indicate that the use of the Arrhenius equation (Logk = LogA -Ea/2.303RT) at high temperatures is valid 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 4 and 5, and the resulting line is extrapolated to room temperature. The $k_{25°C}$ is used to obtain a measure of the stability of the drug under ordinary shelf conditions.

The results of this study seem to suggest that ophthalmic solution is more stable than injectable solution, but by considering the standard error and its effects in calculating the rate constants and expiration date, we can say that the room temperature shelf life of both these products is almost two years.

Table 1

Temperature °C	$k \times 10^3$ (day)$^{-1}$	
	Injectable Formulation	Ophthalmic Drop
60	4.72 ± 0.23	6.00 ± 0.60
70	12.60 ± 0.57	9.30 ± 0.36
80	24.60 ± 1.10	31.00 ± 3.20
90	58.00 ± 1.70	131.00 ± 6.20

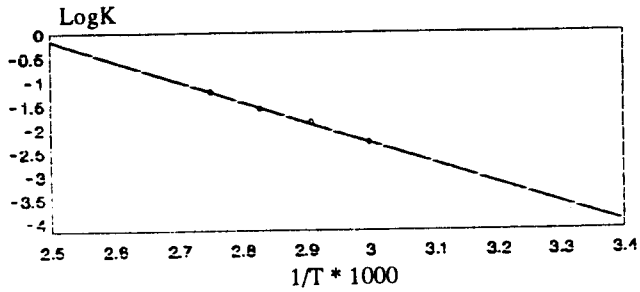

Figure 4. Arrhenius plot for predicting stability of injectable solution at room temperature. A slope of -4.293 ± 0.154, an intercept of 10.56 ± 0.443, a correlation coefficient of -0.998, $k_{25°C}$ of 0.000137 ± 0.0000048 (day)$^{-1}$ and $t_{90\%}$ 25°C of 768 ± 26 (day). Activation energy calculated is 19.64 ± 0.0686 Kcal/mol.

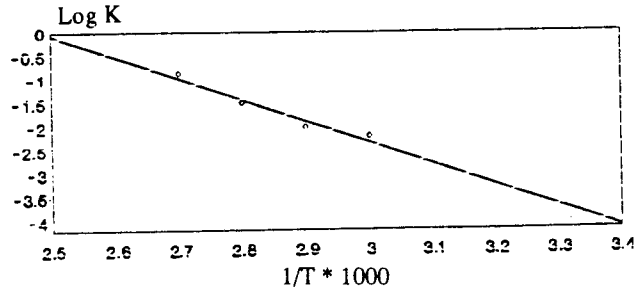

Figure 5. Arrhenius plot for predicting ophthalmic solution stability at room temperature. A slope of -4.54 ± 0.71, an intercept of 11.28 ± 2.03, a correlation coefficient of -.98, $k_{25°C}$ of 0.000107 ± 0.000017 (day) and $t_{90\%}$ 25°C of 979 ± 153 (day). Activation energy calculated is 20.775 ± 3.2 Kcal/mol.

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