Research Article

Stability and Kinetic Studies for the Estimation of Shelf Life of Chloramphenicol, Dexamethasone Sodium Phosphate, and Tetrahydrozoline Hydrochloride Opthalmic Solution

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Accepted on: 20-11-2014; **Finalized on:** 31-12-2014.

ABSTRACT

The purpose of this study is to estimate the shelf life of an ophthalmic solution containing the active ingredients: Chloramphenicol 0.5%, Dexamethasone Sodium Phosphate 0.1%, and Tetrahydrozoline Hydrochloride 0.025%. The shelf life is estimated for a temperature of 5 °C which is the recommended storage temperature for the mentioned product. Accelerated stability studies were conducted under several stress conditions. A validated stability-indicating RP-HPLC method was utilized; degradation kinetics were studied under the following temperature points: 25 °C, 30 °C, and 40 °C; and Arrhenius relationship was applied in the analysis of data and the estimation of the shelf life of the ophthalmic solution at 5 °C.

Keywords: Stability studies, Shelf life, Degradation kinetics, Ophthalmic solution, Chloramphenicol, AMPD

INTRODUCTION

Chloramphenicol is the first broad-spectrum antibacterial to be discovered; it acts by interfering with bacterial protein synthesis and is mainly bacteriostatic. Its range of activity is similar to that of tetracycline and includes Gram-positive and Gram-negative bacteria, Rickettsia spp., and Chlamydiaceae.[1]

Dexamethasone Sodium Phosphate is a water-soluble inorganic ester of Dexamethasone. Dexamethasone is an adrenocortical steroid anti-inflammatory drug.[2] It decreases inflammation by acting within cells to prevent the release of certain chemicals that are important in the immune system. These chemicals are normally involved in producing immune and allergic responses. By decreasing the release of these chemicals in a particular area, inflammation and allergic reactions are reduced.[3]

Tetrahydrozoline (Tetryzoline), a derivative of imidazoline, is found in Over-The-Counter (OTC) eye drops and nasal sprays. It is an alpha agonist and its main mechanism of action is the constriction of conjunctival blood vessels.[4] This serves to relieve the redness of the eye caused by minor ocular irritants.

An ophthalmic solution contains Chloramphenicol 0.5%, Dexamethasone Sodium Phosphate 0.1%, and Tetrahydrozoline Hydrochloride 0.025% is available in the market.[5] It is indicated for keratitis and conjunctivitis acute and chronic infectious, inflammation of the uvea anterior, scleritis, and sympathetic ophthalmia.

According to ICH guidelines, a drug product should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.[6]

The aim of this study is to evaluate the stability of locally made generic ophthalmic solution that contains Chloramphenicol 0.5%, Dexamethasone Sodium Phosphate 0.1%, and Tetrahydrozoline Hydrochloride 0.025% under several temperature points for a duration of 28 days and to estimate the shelf life of the product under the recommended label storage conditions.

MATERIALS AND METHODS

Chemicals and Solutions

Chloramphenicol was obtained from Chemo, Spain. Dexamethasone Sodium Phosphate and Dexamethasone were obtained from Symbiotica, Malaysia. Tetrahydrozoline Hydrochloride was obtained from S.I.M.S, Italy. 2-amino-1-(4-nitrophenyl)propane-1,3-diol (AMPD) was obtained from British Pharmacopoeia Commission Laboratory. Ophthalmic solution that contains Chloramphenicol 0.5%, Dexamethasone Sodium Phosphate 0.1%, and Tetrahydrozoline Hydrochloride 0.025% was purchased from the local market made by Diamond Pharma, Syria. Acetonitrile used was of HPLC grade. All other reagents used in this study were of AR grade. USP purified water was used for making the solutions.

Method of Analysis

A validated stability-indicating RP-HPLC method was utilized for stability assessment of the ophthalmic solution under accelerated conditions. The method can separate all APIs of the ophthalmic solution: Chloramphenicol, Dexamethasone Sodium Phosphate, and Tetrahydrozoline Hydrochloride. In addition to the

degradation products generated from forced degradation studies including Dexamethasone and 2-amino-1-(4-nitrophenyl)propane-1,3-diol (AMPD). Figure 1 shows the chromatogram of standard solution of the analytical method.

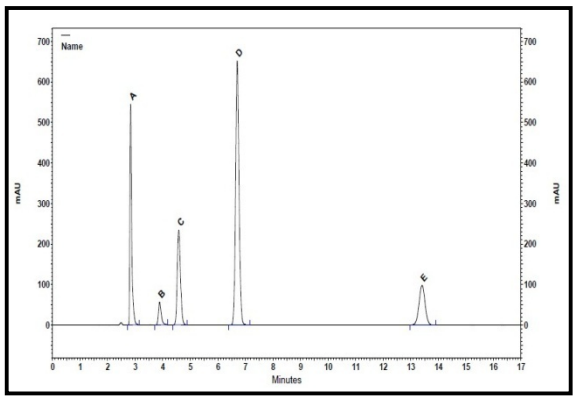

Figure 1: Typical chromatogram of standard solution of the analytical method, (A): AMPD peak, (B): Tetrahydrozoline Hydrochloride peak, (C): Dexamethasone Sodium Phosphate peak, (D): Chloramphenicol peak, and (E): Dexamethasone peak

Stability and Kinetic Studies

The recommended storage condition of the ophthalmic solution is refrigeration (5 °C ± 3 °C).

At the start of the study, the bottles we used were all from the same batch and were 8 months pass the manufacturing date. We confirmed that the bottles were continuously kept under proper storage in a refrigerator. Initial assay were done to confirm the time zero concentrations. The bottles were placed in different stability chambers with the following conditions: (25 °C ± 2 °C/40 ± 5% RH, 30 °C ± 2 °C/65 ± 5% RH and 40 °C ± 2 °C/75 ± 5% RH) for 28 days. Following the protocol, samples were withdrawn at time intervals of 7, 14, 21 and 28 days and analyzed after cooling using the validated HPLC method. The amount of drug degraded and the amount remaining at each time interval were calculated. The order of degradation was determined by the graphical method. Degradation rate constant (K_{deg}) was determined at each temperature point. The Arrhenius plot was constructed between log K and 1/T to determine the shelf life of the ophthalmic solution.

RESULTS AND DISCUSSION

The stability studies showed that Chloramphenicol is the least stable API in the ophthalmic solution. Therefore, the shelf life of the ophthalmic solution was calculated according to Chloramphenicol degradation profile.

Chloramphenicol eye solution should contain from 90.0% to 130.0% of the label amount of Chloramphenicol according to the United States Pharmacopoeia.[7] The British Pharmacopoeia, on the other hand, allows for a smaller tolerance range of 90.0% to 110.0%, and states that AMPD should be less than 8% in the ophthalmic solution.[8]

Table 1 illustrates the degradation of Chloramphenicol at each temperature.

The order of degradation was determined by graphical method at each temperature which was found to be first order (Figure 2) where log % of drug remaining was plotted against time. Degradation rate constant (K_{deg}) was calculated from the slope of the curve at each temperature using the formula[9]:

$$\text{Slope} = \frac{-K_{deg}}{2.303}$$

The Arrhenius plot was constructed between log K and 1/T to determine the shelf life of the ophthalmic solution at 5 °C (Figure 3).

$$\log K = \log A - \frac{Ea}{2.303\,RT}$$

Where **K** is the rate constant, **A** the frequency factor, **Ea** the activation energy, **R** the gas constant (1.987 cal/K/mol), and **T** the absolute temperature in degrees Kelvin.

The value of K at 5 °C (K_5) was obtained by extrapolation of the plot and shelf life was then calculated by substituting (K_5) in the following equation[10]:

$$t_{0.9} = \frac{0.1052}{K_5}$$

The shelf life of the product was calculated to represent $t_{0.9}$ which is the time needed for Chloramphenicol to lose 10% of its initial concentration.

The degradation rate constants at various temperature points and the shelf life of the ophthalmic solution are reported in Table 2.

Table 1: Degradation of Chloramphenicol at each temperature

Time (Days)	Storage conditions	Chloramphenicol		
		Found (mg/mL)	% Remained	log % Remained
0	25 °C ± 2 °C / 40 ± 5% RH	4.961	100.00	2.0000
7		4.927	99.33	1.9971
14		4.873	98.24	1.9923
21		4.827	97.30	1.9881
28		4.811	96.99	1.9867
0	30 °C ± 2 °C / 65 ± 5% RH	4.961	100.00	2.0000
7		4.895	98.68	1.9942
14		4.856	97.90	1.9908
21		4.794	96.65	1.9852
28		4.728	95.32	1.9792
0	40 °C ± 2 °C / 75 ± 5% RH	4.961	100.00	2.0000
7		4.820	97.17	1.9875
14		4.692	94.59	1.9759
21		4.539	91.51	1.9615
28		4.380	88.29	1.9459

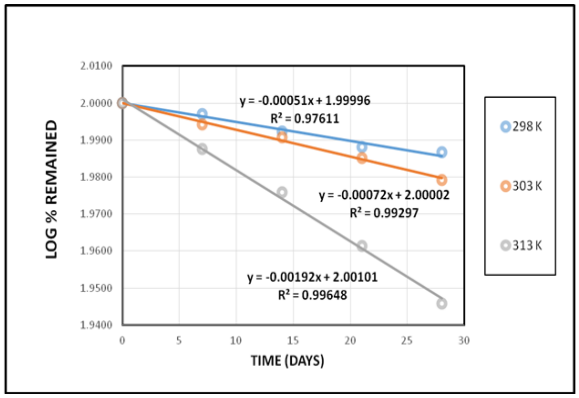

Figure 2: Degradation kinetics of Chloramphenicol at each temperature

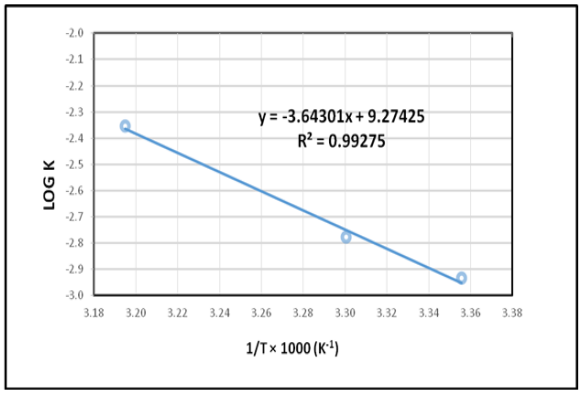

Figure 3: Arrhenius plot for Chloramphenicol

Table 2: Degradation rate constants determined at various temperatures and shelf life of the ophthalmic solution

Temperature (°C)	Temperature (K)	1/T	Slope	K_{deg} ($Days^{-1}$)	log K	$t_{0.9}$ (Days)
25	298	0.00335570	-0.0005082	0.00117038	-2.931671	90
30	303	0.00330033	-0.0007243	0.00166806	-2.777788	63
40	313	0.00319489	-0.0019182	0.00441761	-2.354812	24
5	278	0.00359712	-	0.00014779	-3.830356	713

The results showed that the estimated shelf life of the ophthalmic solution product under investigation was about 23.8 months from the start of the study. However, the study started 8 months passes the manufacturing date. Thus, we can safely say that the ophthalmic product has at least 24 months of shelf life.

Route of Chloramphenicol Degradation

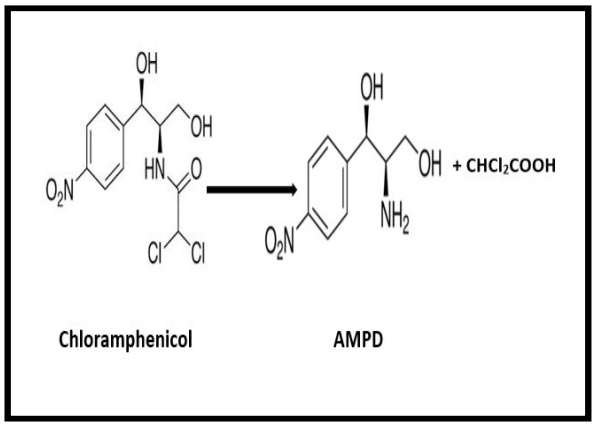

Figure 4: The hydrolysis of Chloramphenicol

Literature[11] shows that the major cause of Chloramphenicol degradation, when protected from light, is hydrolysis resulting in the formation of 2-amino-1-(4-nitrophenyl)propane-1,3-diol (AMPD) and dichloroacetic acid as shown in Figure 4.

In this study, we followed AMPD as the main degradation product of Chloramphenicol and assayed its concentration at each temperature point.

Results are shown in Table 3 and Figure 5.

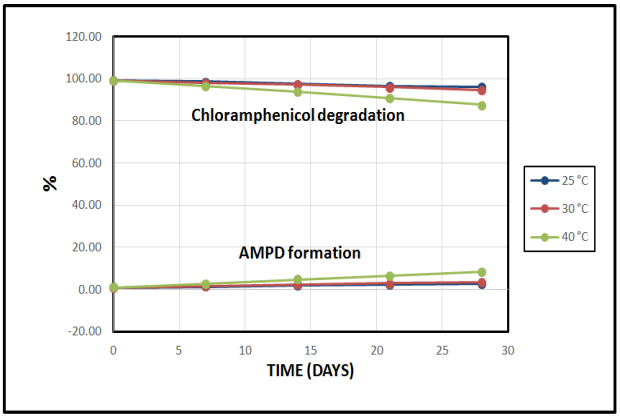

Figure 5: The results of degradation of Chloramphenicol and formation of AMPD at each temperature

Table 3: The results of degradation of Chloramphenicol and formation of AMPD at each temperature

Time (Days)	Chloramphenicol Degradation %			AMPD Formation %		
	25 °C	30 °C	40 °C	25 °C	30 °C	40 °C
0	99.21	99.21	99.21	0.90	0.90	0.90
7	98.55	97.90	96.41	1.31	1.56	2.67
14	97.47	97.12	93.85	1.74	2.23	4.73
21	96.53	95.89	90.78	2.12	2.85	6.49
28	96.22	94.56	87.59	2.51	3.56	8.29

CONCLUSION

The results of this study show that the shelf life of Chloramphenicol, Dexamethasone Sodium Phosphate, and Tetrahydrozoline Hydrochloride local ophthalmic solution at 5 °C is at least 24 months based on 90.0% - 110.0% of the label strength of Chloramphenicol.

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Source of Support: Nil, **Conflict of Interest:** None.