RESEARCH ARTICLE

Preservation Activity of the Selected Preservatives Incorporated in Eye Drop Dosage Forms Marketed in Iraq

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ABSTRACT

Ophthalmic preparation, especially drops, is one of the common pharmaceutical dosage forms intended to be applied to the eye cavity for different pharmacological purposes. Sterility and freeing of microorganisms M.O. are considered one of the most important requirements of the ocular drops, so the manufacturers are very concerned about keeping the sterility of their products. As well as post-marketing pharmaceutical tests must be performed to verify that the eye drop meets the desired properties and withstand the storage condition in pharmacy and during the period after opening the container. The present study aimed to evaluate the preservation activity of different types of preservatives and concentrations utilized in selected eye drops marketed in Iraq

Seven eye drops products were selected and purchased from the Iraqi pharmacies each product was cultured for four weeks at nutrient agar first, then if there is growth identified, the eye drop cultured in MacConkey and mannitol agar for determination of the type of MO.

The addition of benzalkonium chloride (BK) in a concentration of 0.1 mg/mL as in both products (Apisulfa® by API and Orchadexoline® by Orchidia) can guarantee the product protection for 21 days and 14 days respectively.

While the addition of benzalkonium chloride in concentration of 0.2 mg/mL does not protect dexamethasone® eye drop by Eipico. (Xolamol® by Jamjom pharma) results revealed that the 0.75 mg/mL of BK has different and variable protection levels between full to nil protection. Tymer® eye drop shows no growth at all samples cultured in the present study and from all patches in spite of the low concentration of BK. Some samples from apisulfa® and Dexamethasone® as the preservative agent lose their activity totally before 6 months of the expiration date (show M.O. growth at day zero).

Tears natural® II by alcon contains (polidronium chloride) (PQ) in a concentration of 0.001%. Data obtained showed diverse results since samples from one patch show growth of M.O. since the first day while products from different patches show protection for three weeks. Samaphenicol® eye drop by SDI contains a mixed preservative (Thiomersal plus Poly quad (polidronium chloride)) in a concentrations of 0.005 and 0.001%, respectively. Results revealed that all samples survived without bacterial growth for three weeks and one out of three succeeded in staying sterile for the entire period of allowance. However, *Staphylococcus aureus* is only pathogen identified in the eye drops cultured.

Keywords: Benzalkonium chloride, Eye drops, Iraqi Market, Preservative, Thiomersal

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INTRODUCTION

It is essential for most pharmaceutical products to have several additional ingredients (excipients) in addition to active pharmaceutical ingredients (API). These excipients performed specific functions in the prepared dosage form. Eye drops are one of the common pharmaceutical dosage forms intended to be applied for different pharmacological purposes as exerting antibiotic activity or to reduce intra ocular pressure or soothing effect. Whatever the purpose of eye drops, they share the same principle required criteria to be a candidate to administer to ophthalmic cavity among these criteria are isotonicity to ophthalmic fluid, sterility, solubility and non grittiness, and stability (both physical and chemical) in addition to other functions.[1]

Sterility and freeing of M.O. is considered one of the most important requirements of the ocular drops since the introduction of M.O. to eye could be very dangerous and lead to ophthalmic infections that may be hard to be treated.[2] To

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avoid these problems, all manufacturers are very concerned to produce a sterile eye drops with specific sterility assurance level (SAL) also concern in keeping the sterility persists for the entire time of storage and enough time after opening the eye drop container for administration. This is achieved by first sterilizing the produced eye drops and second, adding the preservative to the multiple-dose eye drop.[3]

Sterilization process could be performed either by gamma radiation or electrical beam sterilization and filtration while different types of preservatives (as parabens derivatives) and concentrations are utilized according to the formula composition and formulation[4] and to facilitate the penetration of active compounds into the eye. However, several studies documented significant toxic effects induced by preservatives, especially on the ocular surface. Consequently, most of the ophthalmic medications became progressively available in preservative-free (PF. However, products validation of eye drops in the quality control department of each manufacturing plant is essential and crucial. However, some post marketing test must be performed to verify that the eye drop meets the desired properties and withstand the storage condition both in pharmacy and during the period after opening the container[5] Southlands, and Brighton General hospitals after 7 days' use (341 samples).[6] This study aimed to evaluate the preservation activity of different types of preservatives and concentrations utilized in selected eye drops marketed in Iraq and the effect of storage on their activities with the possibility of bacterial contamination with time after opening the container.

MATERIALS AND METHODS

Product eye drops were selected and purchased from Iraqi pharmacies and their information was collected regarding batch number, manufacturer, and type and concentration of preservative utilized, as shown in Table 1.

A specific procedure was followed for preservative efficiency determination. Several drops from each product were cultured in nutrient agar firstly. If growth is identified, the eye drop was cultured in mackonky and mannitol agar to determine the type of M.O. Biochemical test (as gram stain, coagulase, oxidase and catalyse tests) was performed for better identification of pathogen type. The oxidase test identified the existence of an oxidase enzyme in the bacteria using a redox dye (tetramethyl-p-phenylene-diamine), and the positive result was obtained when the dye was reduced to deep purple color. While the catalase test can differentiate between bacteria that produce enzyme catalase, as staphylococci from non-producing bacteria. The positive result is shown when oxygen gas bubbles were generated after adding hydrogen peroxide (H_2O_2) solution.[7,8]

The above procedure of cultures are performed at intervals of (0, 1, 2, 3, and 4 weeks). In addition, specific procedure was followed for simulation of what happened in real administration of eye drops. This procedure is performed by touching the tip of dropper just prior to inoculation in culture medium touching procedure was performed for all samples except at zero time.

RESULTS AND DISCUSSION

Sterility is one of the most important requirements of ophthalmic preparations and it is one of the major causes of patch rejection in quality control validation and medication recall in post marketing surveillance.[9] However, the efficacy of type of preservative, and concentration incorporated in any formula of eye drop is greatly affected by different factors, including storage conditions studied in the country of origin in which the climate conditions are different from that of Iraq. These possible differences in storage conditions may alter or even lose preservative activity, which can cause serious problems to any patient utilizing these drops.

In the first set of eye drops studied, the addition of benzalkonium chloride (BK) in a concentration of 0.1 mg/mL as in both products (Apisulfa® by API and Orchadexoline® by Orchidia) can guarantee the product protection for 21 days and 14 days, respectively. Bacterial growth was noticed at the fourth week after opening of Apisulfa®. While the addition of benzalkonium chloride in concentration of 0.2 mg/mL (in dexamethasone® eye drops) does not protect the product for the entire period allowed after opening eye dropper as stated by the manufacturer as in case of Dexamethasone® eye drop by Eipico.

Regarding another eye drop namely (Xolamol® by Jamjom pharma) results revealed that the 0.75 mg/mL of BK can protect the product for 28 days (full protection) for products obtained from one patch and failed to exert any protection since day zero in products obtained from other patches. In contrast, product from the same company (Jamjom pharma) namely Tymer® eye drop shows no growth in all samples cultured in the present study and from all patches inspite of the low concentration of BK. These findings could be attributed to the presence of gatfloxacin antibiotic in Tymer® eye drop, which exert additional antibacterial protection.[10]

Product containing benzalkonium chloride show decrease in its preservation activity inversely as the time from production time decrease inspite of the expiration date not being approached. And these findings was shown in the same product of eye drop products but with different production date as shown with both Apisulfa® and Dexamethasone® by Eipico as the preservative agent loses its activity totally before 6 months of expiration date (show M.O. growth at day zero) which indicate a warning sign must be taken in consideration.

Regarding Poly quad (polidron ium chloride) (PQ) preservative, tears natural® II by Alcon contain this preservative in a concentration of 0.001%. Data obtained showed diverse results since samples from one patch show growth of M.O. since the first day while products from different patches show protection for three weeks. In both cases, they are less than the stated period of preservation coverage which is 30 days. A single eye drop sample containing a combination of two preservatives was selected, Samaphenicol® eye drop by SDI, which contains a mixed preservative (Thiomersal plus Poly quad (polidronium chloride)) in concentrations of 0.005 and 0.001%, respectively. Results obtained revealed that all

Table 1: Types and concentrations of pharmaceutical preservative under investigation and results of culture examinations in the specified period of time

Name of selected drop	*Sample tested*	*Production date*	*Expiry date*	*manufacture*	*Patch No.*	*Type of preservative*	*Concentration of preservative (mg/mL)*	*Duration of safley utilization after opening (stated by the manufacurer*	*Examination period -ve means no growth +ve means presence of growth*					*Identified Bacteria*
									0 day	*1 week*	*2 week*	*3 week*	*4 week*	
Apisulfa (Sulfacetamide sodium)	First	12 /2017	12/2020	API Amman pharmaceutical	EG159	benzalkonium chloride	0.1	28 days	-ve	-ve	-ve	-ve	+ ve	*Staphylococcus aureus*
	Second	12 /2017	12/2020	API Amman pharmaceutical	EG159	benzalkonium chloride	0.1	28 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus*
	Third	12 /2017	12/2020	API Amman pharmaceutical	EG159	benzalkonium chloride	0.1	28 days	-ve	-ve	-ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
Dexamethasone	First	4/2019	4/2021	EIPICO	1904544	benzalkonium chloride	0.2	30 days	+ ve	+ ve	+ ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	Second	4/2020	4/2022	EIPICO	2001252	benzalkonium chloride	0.2	30 days	-ve	-ve	-ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	Third	4/2020	4/2022	EIPICO	2001252	benzalkonium chloride	0.2	30 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus (gram stain +ve)*
Orchadexoline	First	5/2018	5/2021	Orchidia	0518143	benzalkonium chloride	0.1	28 days	-ve	-ve	+ ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	Second	6/2020	6/2023	Orchidia	0620110	benzalkonium chloride	0.1	28 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus (gram stain +ve)*
	Third	6/2020	6/2023	Orchidia	0620110	benzalkonium chloride	0.1	28 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus (gram stain +ve)*
Xolamol	First sample	6/2020	6/2022	Jamjoom pharma	XF0047	benzalkonium chloride	0.75	30 days	+ ve	+ ve	+ ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	Second sample	10/2019	10/2021	Jamjoom pharma	WL0190	benzalkonium chloride	0.75	30 days	-ve	-ve	-ve	-ve	-ve	
	Third sample	10/2019	10/2021	Jamjoom pharma	WL0190	benzalkonium chloride	0.75	30 days	-ve	-ve	-ve	-ve	-ve	

	First sample	5/2019	5/2021	Jamjoom pharma	WE0169	benzalkonium chloride	0.05	30 days	-ve	-ve	-ve	-ve	-ve	
Tymer	Second sample	1/2020	1/2022	Jamjoom pharma	XA0143	benzalkonium chloride	0.05	30 days	-ve	-ve	-ve	-ve	-ve	
	Third sample	1/2020	1/2022	Jamjoom pharma	XA0143	benzalkonium chloride	0.05	30 days	-ve	-ve	-ve	-ve	-ve	
	First sample	9/2019	9/2021	Alcon	317909F	Poly quad (polidronium chloride)	0.001%	30 days	+ ve	+ ve	+ ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
Tears Natural II	Second sample	9/2019	9/2021	Alcon	317809F	Poly quad (polidronium chloride)	0.001%	30 days	-ve	-ve	-ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	Third sample	9/2019	9/2021	Alcon	317809F	Poly quad (polidronium chloride)	0.001%	30 days	-ve	-ve	-ve	+ ve	+ ve	*S. aureus (gram stain +ve)*
	First sample	5/2020	11/2021	Samaraa Drug Industries SDI	20 12	Thiomersal + Poly quad (polidronium chloride)	0.005 % + 0.001 %	14 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus (gram stain +ve)*
Samaphenicol	Second sample	4/2020	10/2021	SDI	20 10	Thiomersal + Poly quad (polidronium chloride)	0.005 % + 0.001 %	14 days	-ve	-ve	-ve	-ve	-ve	
	Third sample	4/2020	4/2021	SDI	20 10	Thiomersal + Poly quad (polidronium chloride)	0.005 % + 0.001 %	14 days	-ve	-ve	-ve	-ve	+ ve	*S. aureus (gram stain +ve)*

samples survived without bacterial growth for three weeks and one out of three succeeded in staying sterile for the entire period of allowance by the manufacturing company and this could be attributed to the synergistic effect of both types of preservative[11] and the presence of antibiotic (chloramphenicol) in this eye drop. In addition to this product, only one was manufactured in Iraq and its formula was customized for Iraqi climate so that the preservative does not lose its activity over time. Finally, *S. aureus* is only pathogen identified in the eye drops cultured and they showed biochemical test results as (gram stain +ve, coagulse test +ve, oxidase test –ve and catalase test +ve).

CONCLUSIONS

The efficiency of preservatives in eye drops may decrease after a period of time since production and its inability to exert full protection against M.O., so researchers recommend evaluating eye drops products periodically after production and re-evaluating the type and concentration incorporated in such formulas.

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