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Original article

Quality Control Testing of Chloramphenicol Eye Drops

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ABSTRACT

***Background and aims**. Choramphenicol is one of important therapeutic options for bacterial conjunctivitis. Its eye drop formulation must possess standards of good quality and sterility. The drug is very sensitive to light and temperature and must be stored between 2 and 8 ° C. This study included testing two of the marketed eye-drops formulations of chloramphenicol of two different origins to ensure quality and efficacy of the drugs. **Methods**. The test methodology followed same pharmacopeial tests to evaluate both physical and chemical aspects of the drugs tested. Identification tests and assay were carried out. **Results**. Both products passed the identification tests however, there were different findings and the product from Pakistani company failed to comply with BP limits for the pH range and assay. **Conclusion**. It is of great importance to check on some drugs to see if affected during transport and distribution by testing samples collected randomly from pharmacies.*

Keyword. Effectiveness, Chloramphenicol, Pharmacopoeia.

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الخلفية والأهداف. يعد الكلورامفينيكول أحد الخيارات العلاجية المهمة لالتهاب الملتحمة الجرثومي. يجب أن تتمتع تركيبة قطرات العين بمعايير الجودة الجيدة والتعقيم. الدواء حساس للغاية للضوء ودرجة الحرارة ويجب تخزينه بين 2 و 8 درجات مئوية. تضمنت هذه الدراسة اختبار تركيبتين من قطرات العين المسوقة من الكلورامفينيكول من أصلين مختلفين لضمان جودة وفعالية الأدوية. **الطرق**. اتبعت منهجية الاختبار نفس الاختبارات الدوائية لتقييم الجوانب الفيزيائية والكيميائية للأدوية التي تم اختبارها. تم إجراء اختبارات التعريف والتحليل. **النتائج**. اجتاز كلا المنتجين اختبارات التعريف، ومع ذلك، كانت هناك نتائج مختلفة وفشل المنتج من الشركة الباكستانية في الامتثال لحدود BP لنطاق الأس الهيدروجيني والتحليل. **الاستنتاج**. من المهم للغاية التحقق من بعض الأدوية لمعرفة ما إذا كانت تتأثر أثناء النقل والتوزيع عن طريق اختبار العينات التي تم جمعها عشوائيًا من الصيدليات.

INTRODUCTION

Chloramphenicol is considered as an effective broad-spectrum antibiotic for conjunctivitis in every age [1,2]. Therefore, it is very important to evaluate the concentration as well as physical parameters of such eye preparations. Many pharmaceutical active ingredients are decomposed when exposed to oxygen or light [3]. From the chemical structure of chloramphenicol showed in figure 1, it is clear that this compound is sensitive to moisture, oxygen and light [4].

In addition to chemical stability, there are other quality control tests for ophthalmic preparations such as clarity, pH, identification, uniformity of volume, content uniformity and sterility. All these tests should comply with specifications of IP, BP, USP, JP and Ph throughout the date of their effective shelf-life [5]. Therefore, for each country should demand an

effective and safe product those meeting global pharmacopeial specifications.

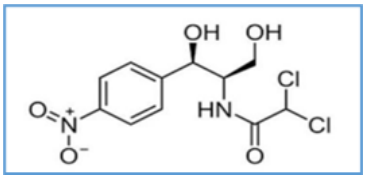

Figure 1: Chemical structure of chloramphenicol.

Ophthalmic formulations of chloramphenicol are available in Libyan pharmacies from different companies as eye ointments and eye drops. This work involved many QC tests for two brands of chloramphenicol eye drops as listed in the following table.

Table1. Preparations of chloramphenicol tested.

Code	Origin	Excipients	Batch	Mf date	Exp date
A	Jordan	Benzalkonium chloride	EI465	5-2023	5-2025
B	Pakistan	N/O	D5256	4-2023	3-2025

Storage and stability of Chloramphenicol

Chloramphenicol eye drops must be stored in refrigerators (between 2°C - 8°C) and away from direct heat and sunlight during course of administration [6]. Moreover, Libya has hot climate (>40°C in summer) so it is possible to expose the product unintentionally to prolonged heat and sunlight leading to decrease in stability and efficacy of the drug. Therefore, government organizations should test chloramphenicol and similar products regularly as a part of post-marketing quality control procedures [1].

Methods for assay of chloramphenicol

There are chemical and microbiological assay methods for evaluating chemical concentrations of chloramphenicol eye products. Chemical methods include capillary electrophoresis, high performance liquid chromatography (HPLC), ultraviolet (UV) spectrophotometers, which are used for the quantitative determination of chloramphenicol. With microbiological assay; it is possible to assess both concentration and biological efficacy of the product in one assay [7].

MATERIALS AND METHODS

Materials

Some of physical tests included use of glassware in addition to a pH meter ((50101212 XS pH 8 PRO, Italy), UV spectrophotometer (Analytik Jena Specord 200, Germany), steam sterilizer (Raypa, UK).

Method

Description

Packaging and labels were checked and observations were listed.

Physical tests

Uniformity of Volume

Content of 3 containers from each brand were examined for measuring volume using a pipette.

Acidity and Alkalinity

pH values were measured using the pH meter. pH values must be 7-7.5 [8].

Identification

Dissolve 10 mg in 2 ml of ethanol (50%). Add 4.5 ml of 1M sulphuric acid and 50 mg of zinc powder and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of sodium nitrite solution, after 2 minutes, 1 g of urea followed by 1ml of 2-naphthol solution and 2 ml of 10M sodium hydroxide; a red colour produced.

Repeate the test without adding the zinc powder; no red colour is produced [8].

Maximum ultraviolet wavelength (λ max)

The solution of eye drop was screened for detecting λ_{max} using the UV spectrophotometer [8].

Assay

Dilute a volume of eye drop containing 25 mg of chloramphenicol to 250 ml with water. Dilute 10 ml to 100 ml with water and measure absorbance of the resulting solution at maximum at 278 nm. Calculate the content of chloramphenicol taking 297as the value of A (1%, 1cm) at the maximum at 278 nm [8].

Sterility

Media for the test is Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria. Mix 29.8g of fluid thioglycollate with 1000ml of distilled water and shake the mixture until dissolved then poured flask. Transfer the bottles to the steam sterilizer.

After transferring 2ml of content of the drop or containers inside Laminar flow hoods to protect the working environment from dust and other airborne contaminants by maintaining a constant, unidirectional flow of HEPA-filtered air over the work area and Laminar flow hoods protects the working environment from dust and other airborne contaminants by maintaining a constant, unidirectional flow of HEPA-filtered air over the work area. To test the culture medium, add an inoculum and Incubate portions of the media for 14 days [8].

RESULTS

Description

The findings were listed in the following table where they made the tested drugs pass the description test.

Table 2. Assessment of description test

Code	Origin	Excipients	Storage	Rx	comment
A	Jordan	Benzalkonium chloride	2-8 °C	Yes	Accepted
B	Pakistan	N/O	2-8 °C	Yes	Accepted

Physical tests

Uniformity of Volume

Although the volume is less than the listed on the package for Code B; it is still complied with limits of IP.

Table 3. Assessment of Uniformity of Volume test

Code	Origin	Stated	Measured	Net volume	Comment
A	Jordan	10 ml	10 ml	100%	comply
B	Pakistan	10 ml	9.7 ml	98.3%	comply

Acidity and Alkalinity

From the listed results for pH values; Code A met the required limits by BP while cod B failed to meet BP standards for pH.

Table 4. Assessment of Acidity and Alkalinity test

Code	Origin	pH	reference	Comment
A	Jordan	7.25	7-7.5	Accepted
B	Pakistan	5.37	7-7.5	Rejected

Identification

The test with zinc powder

Appearance of red colour upon addition of zinc powder was observed with the two drugs tested which confirm the presence of API.

Table 5. Results of test with zinc powder.

Code	Origin	With zinc	Without zinc	Comment
A	Jordan	Red	No red	confirmed
B	Pakistan	Red	No red	confirmed

Maximum ultraviolet wavelength (λ max)

Both products showed to have maximum absorbance at 278 nm confirming the presence of chloramphenicol. The absorbance curves obtained are showed in figure 2.

Table 6. Result of Maximum ultraviolet wavelengthtest

Code	Origin	Reference λ max	Measured λ max	Comment
A	Jordan	278 nm	278 nm	confirmed
B	Pakistan	278 nm	278 nm	confirmed

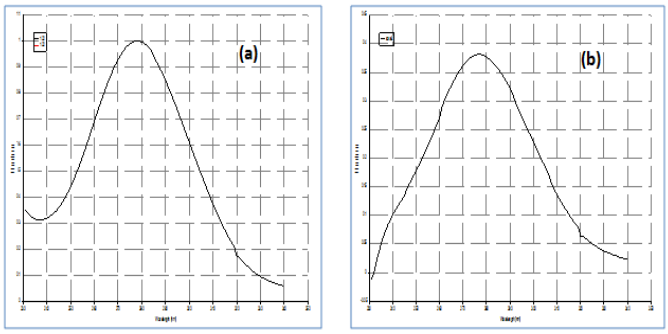

Figure 2: a): Absorption curve for Code A; λmax at 287nm. b): Absorption curve for Code B; λmax at 287nm.

Assay

The results for content uniformity indicated that code B failed to comply with pharmacopeial standards.

Table 7. Assay results; reference values: A = 297, limit = 90-110%.

Code	Origin	Measured A	% Content	comment
A	Jordan	0.3268	110.45%	Accepted
B	Pakistan	0.3388	114.07%	Rejected

Sterility

Both brands had successfully fulfilled the required standards and are devoid of any contamination or presence of microorganisms.

DISCUSSION

Tissues of the eye are very sensitive so it is very important to verify the different qualifications of the ophthalmic preparation along with very clear imprinted information as Rx [9, 10].

The pH of an ophthalmic product is very important. Normal tears have a pH of about 7.4 and show some buffer capacity. Therefore it is predicted that eye drops must be around these values and so the drug code A but not code B[11].

Consistent with the Indian Pharmacopoia (IP); the net volume of the contents of any single container is not less than 91% and not more than 109 % of the labeled amount where the labeled amount is 50 ml or less this test is applicable for eye drops. The results confirmed that both products complied with this test [12].

The purpose of an identification or identity test is to verify the identity of the active pharmaceutical ingredient (API) in the ophthalmic pharmaceuticals. This test should be able to discriminate between compounds of closely related structures that are likely to be present or impurities of similar structure [13]. The two tested products complied with the two identification tests listed in the BP.

This test is designed to determine the strength or content of the API in the ophthalmic pharmaceuticals and is sometimes called a content uniformity test [14]. It is one of the most important tests to accept or reject a finished pharmaceutical product. The obtained values for code A comply with BP limits whereas code B drug did not comply.

Sterility is defined as the absence of viable microbial contamination. Sterility is an absolute requirement of all ophthalmic and intravenous drugs. Asterile ophthalmic formulations could possibly result in serious eye infections that could eventually lead to blindness, especially if the *Pseudomonas aeruginosa* microbe is present. Therefore, ophthalmic formulations must be prepared and packaged in sterile containers [5]. For the tested drugs, both were found to be sterile.

CONCLUSION

Code A drug was found to comply with all pharmacopoeial tests performed whereas code B formulation had failed to comply with some tests such as pH, assay. It is recommended to carry out test of compatibility to the eye on sample code B to reassess validity its pH levels. These findings press toward implying post-marketing quality control to ensure safety, stability and effectiveness of ophthalmic preparations.

Acknowledgments

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Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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