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ORIGINAL ARTICLE

UV-Spectrophotometric Evaluation of the Stability of Ciprofloxacin Eye Drops at Various Temperature Conditions

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ABSTRACT

Ciprofloxacin eye drops, a topical antibiotic for ocular diseases, is commonly obtained "over the counter," and people are not educated about the importance of storage of the medications, thus interfering with their stability. This also leads to the emergence of antibiotic resistance due to the loss of its potency. Since the room temperature in the Southern India is higher (>40°C) during summers, it is possible that there may be some amount of degradation of ciprofloxacin. Hence, the aim of the study was to estimate the effect of different storage temperatures on the potency of Ciprofloxacin at different time intervals. To evaluate the potency of 0.3% Ciprofloxacin eye drops at different storage temperatures for a period of 90 days using UV Spectrophotometer. 28 dropper bottles of 0.3% Ciprofloxacin (10 ml) were purchased from CMC Hospital pharmacy, belonging to the same batch and same brand. After randomization, 7 samples were taken as a baseline and assessed. The remaining 21 samples were divided among 3 groups (groups A, B and C) and stored at different 3 storage conditions (air-conditioned room temperature, room temperature, oven temperature), with 7 samples in each group. Concentrations of ciprofloxacin were evaluated at different time intervals at 3 different storage temperatures (25°C, 30°C, 40°C) using UV Spectrophotometer and analysed for its degradation. The 0.3% Ciprofloxacin eye drop concentration under simulated use conditions was found to be between 90% and 110% of its initial baseline value at different time intervals and three distinct storage settings.

Keywords: Ciprofloxacin; Eye-drops; Antibiotic-resistance; Stability; UV-Spectrophotometer; Fluroquinolones

INTRODUCTION

Antibiotics are the pharmaceutical products that is most frequently fabricated and tampered with. This is most likely a result of their extremely high usage frequency. [1] Numerous variables, including the route of administration, the site of the infection, the presence of substances that interfere with the medication's efficiency, the concentration of the drug in the body, the kind of pathogen, the existence of drug allergies, and the resistance of the microorganism to the drug, affect how effective antibiotic drugs are. Although antibiotic-resistant microbes are becoming a bigger threat to public health, antimicrobial chemotherapy is essential in the battle against infectious diseases brought on by microorganisms. Antibiotic overuse can result in super-infections and contribute to the growth and dissemination of antibiotic resistance. Thus, the measurement of the true concentration of the active ingredient in antibiotic preparation is crucial because of the rise in resistance issues. [2] Antibiotic potency

is a measure of how effective an antibiotic is; even a slight variation in the active ingredient concentration in antibiotic formulations can affect the drug's effectiveness. Accurately measuring the potency and bioactivity of antibiotics is important for addressing the resistance issue and ensuring their safe usage. It is possible to assess an antibiotic's potency chemically and biologically. Microbiological assays are the most efficient and accurate way to ascertain the potency and bioactivity of antibiotics, but chemical methods like capillary electrophoresis, ultraviolet (UV) spectrophotometry, high-performance liquid chromatography (HPLC), and high-performance thin layer chromatography (HPTLC) have been used for the quantitative determination of fluoroquinolones in formulations as well as in human urine and serum. [3]

Stability studies are designed to monitor and evaluate the quality of Active Pharmaceutical Ingredients (API) and Finished Pharmaceutical Products (FPP), assess the drug's potency over time under various environmental conditions (e.g., temperature, humidity) to ensure its effectiveness and safety throughout its shelf life. Certain excipients are capable of degradation after prolonged use or incorrect storage, yet the generation of impurities is typically unmonitored and can have an impact on the stability of drugs. Stability is generally determined by five key factors: chemical, physical, microbiological, therapeutic, and toxicological. The manifestation of instability in different dosage forms can vary and may include drug precipitation, microbial contamination, chemical degradation (for liquid dosage forms, such as elixirs and solutions), organoleptic changes (such as mottling and tackiness) in semisolid and solid dosage forms, and chemical problems (like oxidation and hydrolysis). In FPPs, like solutions, where the API is molecularly distributed, chemical stability is crucial. Degradants can arise over time and cause toxicity in patients in addition to potency loss, therefore, quantifying them is essential for the safety profile of the dosage form. [4]

Review of Literature

A fluoroquinolone antibiotic with broad-spectrum action against the majority of aerobic gram-positive and gram-negative bacteria is ciprofloxacin. It is a quinolone that is quinoline-4(1H)- bearing cyclopropyl, carboxylic acid, fluoro and piperazin-1-yl substituents at positions 1, 3, 6 and 7, respectively, a molecular weight of 331.34 g/mol and its chemical structures is shown in Figure 1.

Among the fluoroquinolone category, ciprofloxacin is typically thought to have the strongest overall antibacterial activity. It is bactericidal and specifically targets DNA gyrase and DNA topoisomerase IV, two significant bacterial topoisomerase enzymes involved in DNA synthesis. In December 1990, the US Food and Drug Administration approved ciprofloxacin as a topical therapy for bacterial corneal ulcers. As of right now, it's the only fluoroquinolone option available

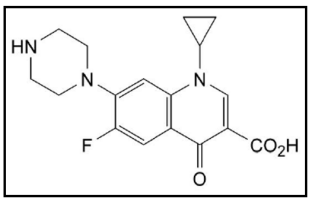

Fig. 1: Structure of ciprofloxacin. Arayne, S. et al. 2009.

on the market in the US for treating corneal ulcers topically. [5] Also, for the first-line treatment of common eye conditions including blepharitis, bacterial keratitis, conjunctivitis, endophthalmitis, dacryocystitis, and orbital cellulitis, topical ciprofloxacin has been hailed as an excellent option and previous researches have demonstrated the effectiveness of a topical antibiotic solution containing 0.3% ciprofloxacin in the treatment of such ocular diseases. [2,6] Over 70% of ocular infections are linked to the following pathogens: *Staphylococcus aureus, Moraxella species, Pseudomonas aeruginosa, Aspergillus species, Fusarium species, Moraxella aureus*, and *Streptococcus pneumoniae*. Even though topical antibiotics can produce larger drug concentrations in ocular tissues, there are growing reports of clinical failure and suboptimal results when fluoroquinolones are used empirically. The overuse of fluoroquinolone antibiotics and the quickly developing antibiotic resistance may be factors in this. [7]

In India, the ciprofloxacin ophthalmic solution 0.3% used as a topical antibiotic for ocular diseases, is commonly obtained by patients by "over the counter" medication and is usually stored at room temperature. The lack of knowledge on the significance of pharmaceutical storage in India causes disruptions to the stability and loss of its potency, ultimately contributing to the rise in antibiotic drug resistance. The manufacture recommends to store the topical antibiotic between 20-25°C. Given that summertime room temperatures in the southern part of India are usually higher than the permitted storage temperature, it is likely that the antibiotic concentration (Ciprofloxacin ophthalmic solution 0.3%) has suffered some degree of degradation. Hence, in this article, the potency of the drug needs to be evaluated by analysing its difference in concentration at different storage temperatures using a UV-Spectrophotometer.

Objective of the Study

To evaluate the potency of 0.3% Ciprofloxacin eye drops at different storage temperatures for a period of 90 days using UV Spectrophotometer.

MATERIALS AND METHOD

Instruments

A Lab India (T-60) UV- Visible spectrophotometer was used for all absorbance measurements.

Materials

The Institution Review Board and Ethics Committee of Christian Medical College, Vellore, India, granted permission to conduct the study in accordance with protocol (IRB Min No. 14904 Dated 12-10-2022). Dropper bottles containing ciprofloxacin 0.3% ophthalmic solution (10 ml) were purchased from the pharmacy at CMC Hospital, belonging to the same brand and batch, to prevent inter-batch variability. They were stored at the pharmacy between 20 and 25 degrees Celsius, when they are claimed to have maximum potency. To prepare the stock and working standards, 99% (TLC grade) pure powder of ciprofloxacin chemical was purchased from Sigma Aldrich Inc. India. The dropper bottles were divided into 3 groups (A, B, C) and stored at three different temperatures, away from sunlight and moisture.

- **Year of Experimentation:** 2023.

Site of study

Department of Pharmacology and Clinical Pharmacology, Christian Medical College, Bagayam Campus, Vellore.

Sample Size Calculation

In a previous study, "Stability and Sterility of Extemporaneously Prepared 0.01% Atropine Ophthalmic Solution in Artificial Tears and Balanced Salt Solution" stability of phenytoin was reported as mean ± SD at different concentrations[8]. The study has reported that at room temperature and in refrigerator, the mean and SD value was 99.44±4.34 and 104.86±2.47. With this value, 5% alpha, 80% power and two-sided test; the current study required 7 ciprofloxacin 0.3% eye drops for each temperature.

Blinding

Code numbers were used to blind the principal investigators, co-investigators and statistician from the sources of the samples; the codes were decoded following analysis. Once the eye dropper bottles containing 0.3% Ciprofloxacin were randomly assigned to groups A (Air-Conditioned room, 25°C), B (Room temperature, 30-32°C), and C (Oven temperature, 40°C), they were numbered 1 – 7. On the day of the experiment, quality control was freshly prepared without disclosing its concentration.

Method

28 dropper bottles of Ciprofloxacin 0.3% eye drops (10 ml) were obtained from CMC Hospital pharmacy. They were then randomised into 4 groups with 7 dropper bottles each, in the ratio of 1:1:1:1. 7 samples from one group were used to estimate their baseline concentrations ("concentration at day 0") using a UV Spectrophotometer. Then, the remaining 21 bottles were grouped and named, A, B, C with seven bottles in each group. All bottles were opened to simulate real-world situations and then stored at different temperature conditions (25°C, 30-32°C, 40°C), protected from direct light. A digital data logger was used to track the storage temperatures.

a. Group A (7 bottles) were stored at 25°C in an air-conditioned room.

b. Group B (7 bottles) were stored at 30-32°C at room temperature (A Digital data logger was used to track the temperatures regularly, and its mean was calculated).

c. Group C (7 bottles) were stored at 40°C in the oven.

An assay of Ciprofloxacin 0.3% eye drops by UV Spectrophotometer was done based on the method developed by Safila Naveed and Nimra Waheed.[9] After 7 days of storage, 7 samples (1 ml from each dropper bottle) from each group were used to measure the concentration of ciprofloxacin 0.3% eye drops at that particular temperature [AC (25°C), room temperature (30-32°C), oven (40°C)]. The concentration of ciprofloxacin eye drops at 3 different temperatures was also estimated at 14, 30, 45 60, and 90 days. The difference in the initial concentration of ciprofloxacin (Baseline) and concentration of ciprofloxacin at 7, 14, 30, 45, 60 and 90 days was analyzed by a UV Spectrophotometer, at the 3 different storage temperatures. Multiple comparisons were done at each time interval for different temperatures. Follow-up values were expressed in terms of the percentage of initial values, and Paired t-test was used to analyse the difference between the values obtained. The flowchart of the methodology is given in Figure 2.

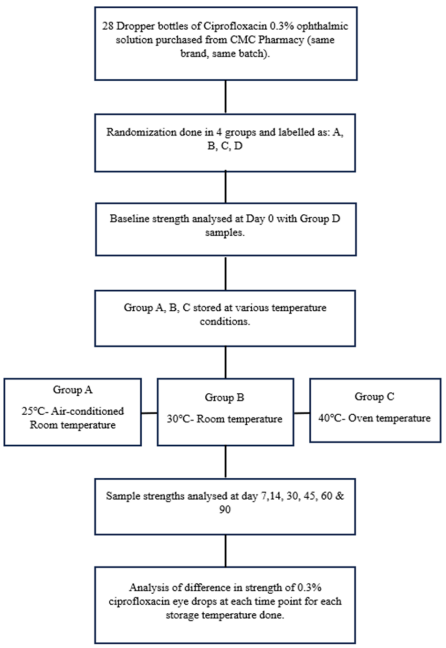

Fig. 2: Methodology Flowchart

Assay

- **Preparation of Stock:** Distilled water was utilized as the solvent to quantify ciprofloxacin, as it is easily soluble in it. The pure powder of ciprofloxacin, purchased from Sigma Aldrich Inc. India was used to construct the stock standard. 10 ml of ciprofloxacin stock was freshly made on the day of the experiment at a concentration of 1 mg/ml. Four working standards (2, 4, 6, and 8μg/ml) were made using the stock standard previously indicated.
- **Determination of wavelength of maximum absorption:** The UV spectrophotometer was used to scan the 20 μg/ml solution made from the previously mentioned stock in the 200–800 nm range. The wavelength of maximum absorption, or lambda max, was discovered to be 274 nm.
- **Preparation of Quality Control (QC):** One known concentration (5μg/ml) was prepared and used for the Quality Control aliquot to ensure the accuracy of the methodology.
- **Preparation of sample aliquots:** Samples from group A (Air-Conditioned Room, 25°C) were taken, with 1 ml from each 0.3% ciprofloxacin eye dropper bottle and 1mg/ml stock was prepared for each sample. Then, from the above stock an unknown concentration was prepared. The same procedure was repeated for groups B (Room temperature, 30-32°C) and C (Oven, 40°C) across 7, 14, 30, 45, 60 and 90 days. For improved precision, each sample aliquot was measured twice and averaged.
- **Estimation of standards, QC and Unknown samples:** The working standards, quality control and unknown were subjected to estimation using UV-Visible Spectrophotometer. At first, the absorbance of a blank sample was read at 274 nm to nullify any error. Then, 3.5 ml of each aliquot was added to the quartz cuvette and sequential spectrophotometric measurements were noted. An absorbance-concentration graph was plotted with the standards and the coefficient of correlation was computed for the standard curve. Further, quality control and the unknown concentration were determined by using the straight-line equation (**y = mx + c**).
- **Linearity and Range:** We evaluated the quality of our calibration curve using the correlation coefficient. Within the concentration range of 2.0–8.0 μg/ml, the samples' absorbance was linear, with a correlation coefficient (R^2) higher than 0.998.
- **Validation of standard curve:** Using the quality control that was previously indicated, the validity of the calibration curve was verified. Quality control in this experiment was made from a different stock standard. A calibration curve was deemed faulty if its quality control value differed by more than 10% from its actual concentration.

Statistical Analysis

The strength of the drug was considered using the given formula:

Strength of the drug = [Observed Quantity/Label Quantity] x 100

According to the 2011 WHO Report, the permitted range of strength is between 90% and 110% of label values; samples were deemed to have degraded/ unsatisfactory if the active pharmaceutical ingredient (API) concentration was outside of this range. [10] Descriptive analysis was done to measure the mean concentrations of 7 samples at baseline (day 0) and at different storage conditions (Air-conditioned room temperature, room temperature, Oven temperature) at different time intervals (7, 14, 30, 45, 60, 90 days).

The minimum and maximum mean concentrations at different time intervals were compared to the baseline mean concentration value and expressed as a percentage of the baseline value, which represented the strength of the drug. This was then analysed to determine if the strength of the concentrations lay within the permitted range of 90%-110%, according to the 2011 WHO Report, to eliminate the degradation possibilities.

RESULTS

Throughout the investigation, there was no bacterial contamination in any of the simulated 0.3% Ciprofloxacin eye drop solutions that were prepared. There was no change in the material's physical appearance (no precipitation/ discoloration), and the pH levels stayed almost the same.

- **Air-Conditioned room temperature (25°C) storage**

The maximum mean ± SD concentration at air-conditioned room temperature storage was seen at day 90 (6.5 mg ± SD0.06), and the minimum mean ± SD concentration was seen at day 30 (6.05 mg± SD 0.10). The strength of their concentration was found to be 104.57% & 97.43% respectively. As this happened to be within 90% - 110% of the initial value, there seems to be no degradation of 0.3% ciprofloxacin eye drops at any time interval when stored in an air-conditioned room (25°C).

- **Room temperature (30°C) storage**

The maximum mean ± SD concentration at room temperature storage was seen at day 60 (6.51 mg ± SD 0.06 and the minimum mean ± SD concentration was seen at day 45 (5.73 mg± SD 0.17). The strength of their concentration was found to be 105% and 92.1% respectively. As this happened to be within 90% - 110% of the initial value, there seems to be no degradation of 0.3% ciprofloxacin eye drops at any time interval when stored at room temperature (30°C).

- **Oven temperature storage (40°C)**

The maximum mean ± SD concentration at oven temperature storage was seen at day 90 (6.44 mg ± SD 0.13), and the minimum mean ± SD concentration was seen at day 30 (5.95 mg ± SD 0.101). The strength of their concentration was found to be 103.67% and 96.02% respectively. As this happened to be within 90% - 110% of the initial value, there seems to be no degradation of 0.3% ciprofloxacin eye drops at any time interval when stored at oven temperature (40°C).

Thus, in simulated use condition, the 0.3% Ciprofloxacin eye drop concentration was within 90% to 110% of initial value after 3 months at different storage conditions.

Overall, in Figure 3, the Box plot shows the concentrations of ciprofloxacin eye drops (mg), at different storage conditions (Air-Conditioned room temperature (25°C) storage, Room temperature (30°C) storage & Oven temperature storage (40°C).

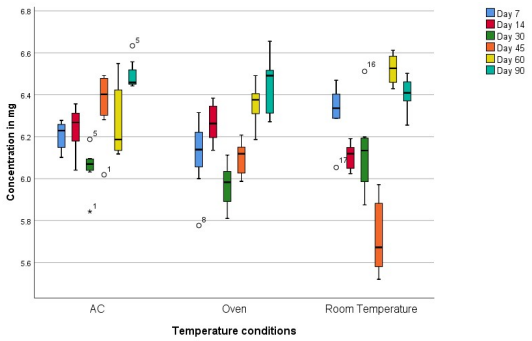

Fig. 3: Box plot: Concentration of ciprofloxacin eye drops (mg), at different storage conditions

DISCUSSION

According to a study, after assessing the variability in the content of ciprofloxacin eye drops, it was found that 20% of those generic eye drops, purchased 'over the counter' in India were found to be under-potent. The antibiotic concentration in several formulations was low enough to potentially affect clinical outcomes and perhaps select resistant isolates in specific patients.[11] In a different study examining the photostability of Lomefloxacin and Ciprofloxacin in various formulations, it was shown that the Ciprofloxacin eye drops deteriorated after just one month of exposure to direct light. Due to photodegradation, the concentration of the active ingredients decreased significantly and statistically when the eye drop was exposed to light in both the amber and plastic containers.[12] The UV spectrophotometric approach is a basic, rapid, precise, and economical way to test for the stability of pharmaceutical drugs. Therefore, in order to determine the potency of 0.3% Ciprofloxacin eye drops in real-world simulation, this study used a UV Spectrophotometer to measure its stability at various storage conditions and time intervals.

According to our study findings, the 0.3% Ciprofloxacin eye drop concentration at various time intervals and 3 different storage settings was between 90% to 110% of its initial value under simulated use conditions. Thus, we conclude that the research groups were within the standard WHO-designated limits, and there was no evidence of degradation noticed over a period of 3 months.

Nonetheless, the general public needs to be informed about drug stability. Degradation impurities have the potential to induce a loss of efficacy and produce negative consequences, which can impact the safety and efficacy of the therapeutic product. Therefore, in order to guarantee the quality and safety of pharmaceuticals, awareness should be raised about achieving the chemical and physical stability of medications. Also, how important it is to adhere to the specific handling and storage guidelines listed in the package insert.

This spectrophotometer-based approach to measuring tablet strength is unquestionably less expensive than more costly, labour-intensive, and skill-intensive processes like LCMS or HPLC. The medications that are given to the patient can be quantified using this simple technique to rule out substandard dosage. It is crucial to guarantee that all patients take medications of standard quality in order to avoid sub-therapeutic reactions, which can lead to treatment failure and drug resistance.[13]

Limitations of the study:

A Reliable, efficient, and cost-effective method for quantitative analysis in stability testing research is the UV spectrophotometric method. However, the most accurate, sensitive, and validated approach for quantifying pharmaceuticals and substances is high-performance liquid chromatography (HPLC). Thus, the drug formulation used in our study, if found to have undergone degradation (Strength of the drug concentrations <90% or >110%), would be confirmed with a more accurate and sensitive analytical technique (HPLC).

CONCLUSION

The stability testing of ciprofloxacin eye drops using UV-spectrophotometry offers valuable insights into the degradation kinetics and shelf-life of the formulation. By monitoring the absorbance of ciprofloxacin at specific wavelengths over time, we were able to assess its stability under various storage conditions. The results obtained from UV-spectrophotometric stability testing in our study states that, in simulated real-world situation, the 0.3% Ciprofloxacin eye drop concentration was within 90% to 110% of initial value after 3 months at different storage conditions. Therefore, we draw the conclusion that, over the course of three months, there was no evidence of

degradation and that the study groups were operating within the standard WHO-approved parameters. This serves as crucial data for regulatory compliance and quality assurance in pharmaceutical manufacturing. Overall, UV-spectrophotometry is a reliable and cost-effective method for evaluating the stability of ciprofloxacin eye drops, providing essential information for ensuring the safety and efficacy of the product throughout its shelf life.

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Declaration of Competing Interests

All the authors declare that there is no competing interest.

Author's contributions

The idea was conceived, and study was designed by MS. NJJ and MH did the main laboratory work under MS's and AK's guidance. AK, GSD, NC, MH assisted in overall implementation of the study. NJJ prepared the first draft. All the authors gave critical inputs and revised the draft. The final draft was approved by all authors. Each author agrees to be accountable for all aspects of the research work.

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