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Effect of sunlight, moisture, temperature and ultraviolet radiation on the quality control parameters of ciprofloxacin tablet formulation

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Abstract

This work assesses the stability and quality of Ciprofloxacin hydrochloride tablets after subjection to accelerated stability conditions of sunlight, temperature of 40±1^{0}C, 75% relative humidity, and UV light of 365 nm for 4 hours each day. This study was performed at time zero and at 4-day intervals for a period of 45 days (that is, days 0, 4, 8, 12…40, 44) according to the International Conference on Harmonization (ICH) accelerated aging conditions and the results obtained were subjected to statistical analysis. The results showed that with increasing time there was a gradual reduction in the dissolution rate with the tablets exposed to all four storage conditions failing the test on day 44 where they had less than 80 % release of the label claim. For content of Ciprofloxacin Hydrochloride, only those tablets exposed to UV light passed the test for all 44 days as they had a minimum of 96.83 % content on the 44th day. At day 0, all the tablets assayed passed this test, having a ciprofloxacin content of 99.43 %. For those tablets subjected to the other storage conditions including temperature of 40±1^{0}C, 75 % relative humidity and sunlight, they had ciprofloxacin content of 70.22 %, 71.50 %, and 78.36 % respectively. The results further, indicated that the storage conditions used in the study had a greater impact on the dissolution behavior and content of the Ciprofloxacin tablets than they did on the physical stability (hardness, uniformity of weight, disintegration).

Keywords: Accelerated Stability Conditions, Stability, Quality, Ciprofloxacin Hydrochloride, Tablets.

Introduction

Ciprofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration and their availability in both oral and intravenous formulations. Among the flouroquinolones however, Ciprofloxacin is the most widely used of the second generation quinolone antibiotics and is one of the most potent [4]. It is very effective, especially used as a better alternative when most antibiotics have failed or in cases of allergic reactions to those other first-line antibiotics. For these reasons, it is imperative that evaluation of the safety and quality of the drug be done especially in this part of the world where adequate measures do not exist for proper management and storage of medications as specified in the official books [1]. Most drugs are not properly stored after they have left the manufacturers - they may be exposed to adverse conditions during shipping and when in possession of distributors and consumers, so much so that when it gets to the end users it may become ineffective and does not meet its therapeutic requirements. Therefore, frequent post market surveillance of drugs such as that undertaken in this study are crucial to protect public health especially in developing countries where counterfeit and substandard drugs have become a major challenge to health care services. Post market surveillance or monitoring involves all activities undertaken to obtain more data and information about a product after it has been granted marketing authorization and made available for public use.

In this study, it was assumed that previous studies must have been carried out prior to release of the tablets and so the duration of this study was not as long as 6 months as recommended by ICH. Hence, this work was carried out basically to confirm the stability of the already 'qualified' Ciprofloxacin tablets and where necessary, give suggestions based on the results obtained.

Literature survey reveals that several investigations on the pharmaceutical quality of Ciprofloxacin tablets after subjection to accelerated stability conditions have been carried out, mainly with respect to the dissolution profiles and content uniformity. Certainly, numerous studies have been conducted on this drug, since it is one of the most prescribed antibiotics and the incessant saturation of the therapeutic markets with different formulations of Ciprofloxacin. Hence, the need for such extensive studies. However, none of these investigations has been reported with respect to the other quality control parameters used to assess the stability of a solid oral drug dosage form such as hardness, disintegration, uniformity of weight, all of which are employed in this study to evaluate the stability of the Ciprofloxacin tablets that have been subjected to accelerated storage conditions such as sunlight, UV light, temperature and moisture. These quality control tests are equally

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important and necessary in evaluation of tablets especially with regards to the physical and chemical stability of drugs.
Also this research work is unique because whilst the two temperature and humidity are the basic conditions required to carry out such stability studies as stated by ICH guidelines, this study includes two more stress conditions such as exposure of the tablets to both sunlight and UV light. Stability studies should include testing of the attributes of those drug substance that are susceptible to change." In this case, Ciprofloxacin is known for its photo reactivity and so inclusion of sunlight and UV light as part of the accelerated storage conditions gives an added advantage.

Materials and Method

Reagents and samples

Ciprofloxacin hydrochloride tablets (Ciproxamed) purchased from a retail pharmacy outlet in Nigeria containing 500 mg Ciprofloxacin Hydrochloride, Pharmaceutical grade ciprofloxacin powder from ACT GEN pharma, India, analytical grade hydrochloric acid, distilled water. All tests were performed within products' expiration dates.

Apparatus and Equipment

ERWEKA hardness tester (Model-TBH 100), ERWEKA dissolution apparatus (Model-DT 600), ACCULAB ALC-210.4 electronic balance, ERWEKA disintegration tester-ZT x 20, JENWAY 6405 UV/VIS spectrophotometer, volumetric flasks, glass test tubes, glass funnels, beakers, measuring cylinders, thermometers, oven.

Method

The following quality control parameters were employed in the assay- weight uniformity, tablet hardness, disintegration, dissolution and content of active ingredient tests according to specifications given in both the British Pharmacopoeia (BP) 2012 and United States Pharmacopoeia (USP) 200. The materials were tested at established time intervals with specific methods of assay in order to distinguish the degradation from inter assay variation.

Exposure to the accelerated stress conditions

The Ciprofloxacin tablets (equivalent to 500 mg of Ciprofloxacin hydrochloride) were exposed to the following stress conditions:-
TEMPERATURE: The ciprofloxacin tablets (700 tablets equivalent to 70 packs as they come in a pack of 10 tablets) were kept at 40 ± 1^0C in an oven for a total of 45 days. Samples from this batch of the ciprofloxacin tablets were taken at 4-day intervals; on days 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and analyzed using these quality control tests-uniformity of weight, hardness, disintegration, content of active ingredient and dissolution test.
SUNLIGHT: 700 ciprofloxacin tablets were exposed to adequate sunlight in clean and dry glass trays without being affected by rainfall and moisture for a total of 45days. Samples were taken at 4-day intervals as described above and the same quality control tests mentioned above were carried out on these samples.
MOISTURE: 700 ciprofloxacin tablets were kept shielded from sunlight but properly exposed to moisture in the environment, with a relative humidity of about 75% for a period of 45 days in total. Samples were taken at 4-day intervals as described above and the same quality control tests were used to evaluate the stability of the tablets after exposure to this stress condition.
UV LIGHT: 700 ciprofloxacin tablets were exposed for 4 hours each day to UV light of 365 nm for a total of 45 days. Again, as with the other stress conditions mentioned above, samples were taken at 4-day intervals and the same quality control tests were carried out on those samples to evaluate their stability after exposure to the stress condition.

Preparation of standard stock solution

Standard solution of Ciprofloxacin hydrochloride was prepared by accurately weighing 10 mg of pharmaceutical grade Ciprofloxacin hydrochloride powder and dissolving in 50 ml of distilled water in a 50 ml volumetric flask to obtain a stock solution of 200 µg/ml.
1 ml of this solution was transferred to another volumetric flask and diluted to 10 ml to get a 20 µg/ml solution.

Determination of wavelength of maximum absorption

The 20 µg/ml solution prepared as described above was scanned in the UV spectrophotometer in the range of 260-280 nm and the wavelength of maximum absorption (lambda max) was found to be 275 nm.

Determination of standard curve (Beer's calibration curve)

From the 20 µg/ml stock solution, other concentrations were derived by conducting serial dilutions whereby 1 ml, 2 mls, 3 mls, 4 mls, 5 mls, 6 mls, 7 mls, 8 mls, 9 mls were withdrawn from the 20 µg/ml solution and diluted to 10 mls each time with distilled water to give their respective concentrations- 2, 4, 6, 8, 10, 12, 14, 16, 18 µg/ml.
These solutions of different known concentrations were scanned at 275 nm using UV spectrophotometer and their absorbance gotten. This was done in triplicates for each concentration.
The calibration curve was plotted as concentration against absorbance mean to generate an equation:
$y = mx.$ (1)

Content of active ingredient

Every fourth day, twenty tablets in total are randomly selected from each of the above stated stress conditions, weighed individually and the mean weight calculated. The tablets are crushed to a fine powder and the mean weight is collected into a 500 ml volumetric flask and dissolved in distilled water up to 500 ml mark. 1 ml of this solution is withdrawn into a 100 ml volumetric flask and further diluted to 100 ml. 5 ml of this second solution is then scanned in the UV spectrophotometer at 275 nm to get its absorbance. The y = mx equation generated from the calibration curve (where y refers to response, m-the slope and x-concentration) was used in quantifying the actual concentration of ciprofloxacin hydrochloride present in each drug sample solution. Hence the amount of Ciprofloxacin hydrochloride present in each tablet was calculated and compared to the label claim. The BP 2009 specifies an acceptable drug content range of 98 – 102 %.

Apart from those methods mentioned in the official books such as HPLC, titrimetry, several ways of measuring the content of Ciprofloxacin in tablets have been used, such as UV spectrophotometry [2-5]. Most of the analytical methods for the determination of Ciprofloxacin employ HPLC. It is also the official method in the United States, Pharmacopoeia, British Pharmacopoeia and Brazilian Pharmacopoeia. But other simple and rapid methods such as UV spectrophotometry for the determination of ciprofloxacin have been developed and validated through the parameters of linearity, accuracy, precision, limit of detection, limit of quantitation, specificity, and robustness according to ICH guidelines and are described in the literature [6]. This is especially important in small laboratories and in places where there are no sophisticated and expensive instruments. This method has been successfully applied to the determination of Ciprofloxacin in solution. This research employs the use of UV spectrophotometry which have also been successfully employed in others countries as mentioned earlier. Thus, the procedure is suitable for the determination of Ciprofloxacin HCL as a routine method with adequate accuracy.

Weight uniformity test

This test was performed as described in the British Pharmacopoeia (2009). The weight of twenty tablets randomly selected from each of the above stated stress conditions were individually weighed using an electronic balance. The mean tablet weight and standard deviation were calculated. This test was repeated at 4-day intervals for each of the stress conditions outlined earlier.

Hardness test

The hardness of 10 tablets selected randomly from each of the above mentioned conditions was determined using an ERWEKA hardness tester. The mean hardness and standard deviation were calculated. The test was repeated at 4-day intervals for each stress condition.

Disintegration test

The ERWEKA disintegration test apparatus was used based on the British Pharmacopoeia 2009 method. The disintegration medium was 0.1N HCl filled to 700 mls in the vessel maintained at 37 ± 0.5^0C. Six tablets from each of the above mentioned conditions selected randomly were used for the test (which was carried out at 4-day intervals). The disintegration time was taken as the mean time needed for the tablets to break into particles small enough to pass through the screen into the disintegration medium.

Dissolution test

ERWEKA dissolution apparatus was used, employing the British Pharmacopoeia 2009 method. Vessels containing 900mls of water as the dissolution medium were used for this test. The vessels were immersed in a circulating water bath maintained at 37±0.5^0C and allowed to equilibrate. Once the desired temperature was attained, the tablets were added to the center bottom of the vessel and the paddle was rotated at 100 rpm. 10 mls of the dissolution medium was withdrawn from each of the vessels using a pipette after 10, 20, 30, 40, 50 and 60 mins and an equivalent amount of the dissolution medium was immediately introduced as a replacement each time. The samples were filtered and the actual concentrations were determined by measuring the absorbance at 275 nm using a UV spectrophotometer. Using the standard curve already prepared with the reference standard, the percent of drug released was determined for the six tablets obtained from each of the stress conditions they were subjected to. The mean was calculated for each of the conditions. The test was carried out at 4-day intervals for each of the conditions.

NOTE: All the quality control tests were performed on the Ciprofloxacin tablets at day 0, prior to their exposure to the four stress conditions outlined earlier, to serve as a baseline so that the rate and extent of degradation can be properly monitored or determined.

Results and Discussion

A summary of the results of weight uniformity, hardness and content of active ingredient, disintegration and dissolution tests are presented in Figures 1-7 and Table 1.

Uniformity of weight

The tablet weight uniformity for the ciprofloxacin tablets studied are within acceptable limits. The British Pharmacopoeia 2012 specifies that for tablets weighing 324 mg and above, weights of not more than two tablets should deviate from the average weight by more than 5 %. Therefore, all the ciprofloxacin tablets exposed to the four accelerated storage conditions of temperature, humidity, sunlight and UV light passed the test for the period of their exposure (throughout the stability study which lasted for 45 days). The maximum deviation of 4.08 % occurred amongst those tablets exposed to moisture on day 36 of their exposure, yet it still complied with the specifications. For the other storage conditions- temperature, sunlight, UV light, they had maximum deviations of 3.16 %, 3.09 % and 2.06 % respectively at the end of the evaluation.

Table 1: Results for uniformity of weight of ciprofloxacin hydrochloride tablets subjected to the four accelerated stability conditions (mean weight presented in mg)

DAYS	SUNLIGHT	MOISTURE -75% RELATIVE HUMIDITY	TEMPERATURE - 40 ± 1^{0}C	UV-LIGHT 365nm for 4hours daily
0	0.97 ± 0.02	0.97 ± 0.02	0.97 ± 0.02	0.97 ± 0.02
4	0.97 ± 0.03	0.97 ± 0.01	0.96 ± 0.02	0.97 ± 0.01
8	0.96 ± 0.02	0.97 ± 0.01	0.96 ± 0.02	0.97 ± 0.01
12	0.96 ± 0.02	0.97 ± 0.02	0.96 ± 0.01	0.97 ± 0.01
16	0.96 ± 0.02	0.97 ± 0.02	0.96 ± 0.01	0.97 ± 0.02
20	0.96 ± 0.02	0.98 ± 0.02	0.95 ± 0.02	0.97 ± 0.02
24	0.96 ± 0.02	0.98 ± 0.02	0.95 ± 0.02	0.97 ± 0.01
28	0.96 ± 0.02	0.98 ± 0.03	0.95 ± 0.02	0.97 ± 0.01
32	0.96 ± 0.02	0.98 ± 0.04	0.95 ± 0.03	0.97 ± 0.01
36	0.96 ± 0.01	0.98 ± 0.04	0.95 ± 0.01	0.97 ± 0.01
40	0.96 ± 0.01	0.98 ± 0.03	0.94 ± 0.02	0.97 ± 0.02
44	0.96 ± 0.01	0.98 ± 0.03	0.94 ± 0.02	0.97 ± 0.02

Each value represents mean weight ± standard deviation

There were some significant changes in weight however, especially for those tablets subjected to sunlight, moisture and temperature. A decrease in weight was observed with increasing time for those tablets exposed to temperature of 40 ± 1^{0}Cand sunlight (a slight decrease though), while for those subjected to moisture an increase in weight was observed. Exposure of the tablets to accelerated storage conditions primarily contributes to atmospheric moisture absorption as demonstrated by tablet weight gain. Generally, uniformity of weight serves as a pointer to good manufacturing practices as well as amount of active pharmaceutical ingredient contained in the formulation.

Hardness test

The mean values obtained for tablet hardness are also within acceptable range. A force of about 4 kg force is the minimum requirement for a satisfactory tablet [7]. The ciprofloxacin tablets under study passed the hardness test for all the days they were subjected to the four accelerated storage conditions as they still had a mean hardness of over 4 kg force at the end of the evaluation (see figure 1). Hardness test assesses the ability of the tablets to withstand handling without fracturing or chipping [7]. It can also influence disintegration and hence dissolution because the harder a tablet, the more time it takes to disintegrate. Generally, there was a decrease in tablet hardness of the ciprofloxacin tablets with time in this order Temperature < Moisture < Sunlight < UV light , with those exposed to temperature of 40 ± 1^{0}Chaving the least value of 4.59 kg force whilst those exposed to UV light had the highest value of 7.85 kg force at the end of the evaluation.

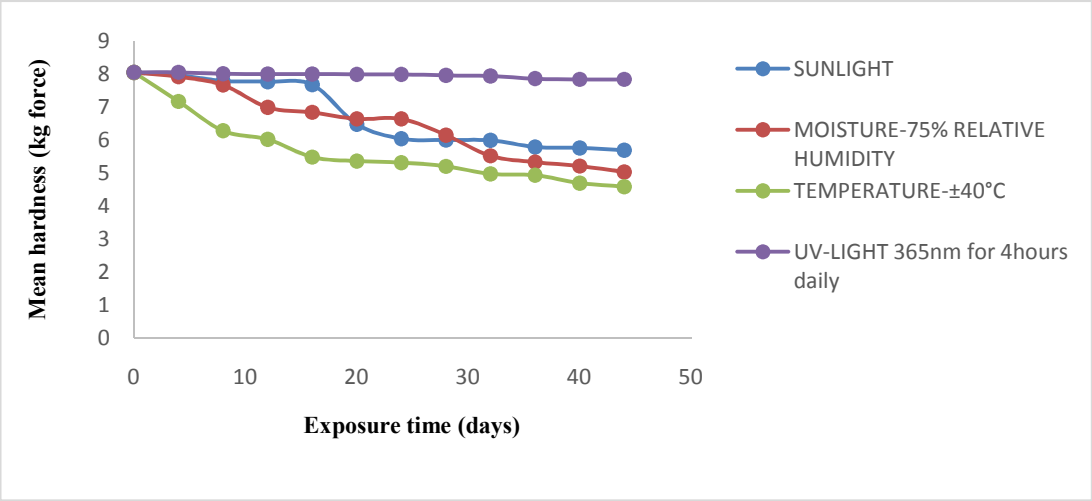

Figure 1: A plot af mean hardness (kg force) against exposure time (days) for ciprofloxacin tablets subjected to accelerated stability conditions

Disintegration test

The British Pharmacopeia 2012 specifies that for uncoated tablets, disintegration time should be within 15 mins. In accordance with this the ciprofloxacin tablets exposed to all four accelerated storage conditions passed the disintegration test for the period of the study (figure 2).

Those tablets exposed to moisture showed a decrease in disintegration time as the number of days increased, starting with a mean disintegration time of 1.15 mins on day 0 and ending on day 44 with a mean disintegration time of 0.40 mins. This was in contrast to those tablets exposed to temperature of 40 ± ^{0}C where their mean disintegration time increased with increasing number of days, starting with a mean disintegration time of 1.15 mins on day 0 and ending with 3.30 mins on day 44. Those exposed to UV light and sunlight had little or no changes in their disintegration time, both starting with a mean disintegration time of 1.15 mins on day 0 and ending on day 44 with 1.09 mins and 1.02 mins respectively.

Disintegration is a crucial step in drug absorption and could at times be directly related to dissolution and bioavailability of a drug. Usually, the higher the tablet hardness, the slower the disintegration and hence dissolution rate [8]. Therefore, it would have been expected that those tablets exposed to UV light which had the highest value for hardness after the period of evaluation would also have the highest disintegration time and those exposed to temperature the fastest. This perceived discrepancy may be as a result of the effect of different storage conditions employed, on the excipients used in each formulation.

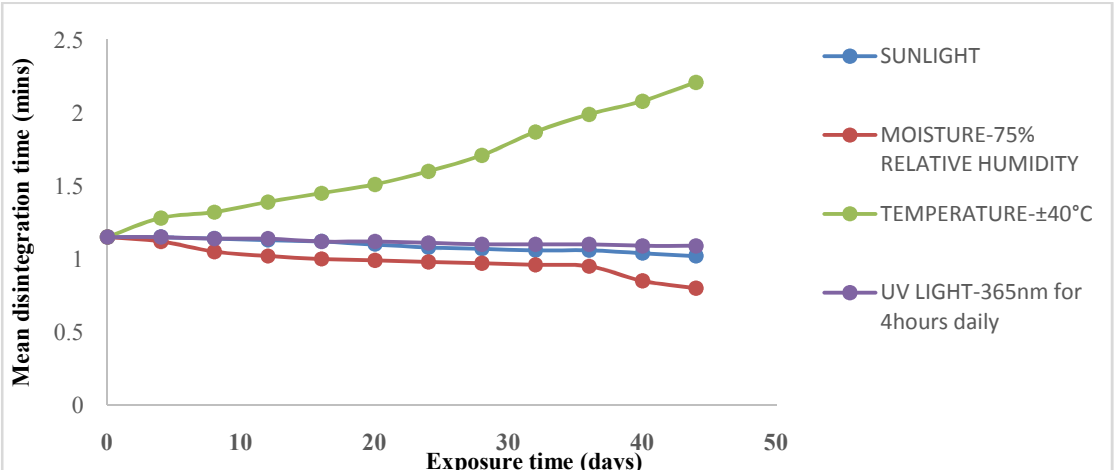

Figure 2: A plot af mean disintegration time (mins) against exposure time (days) for ciprofloxacin tablets subjected to accelerated stability conditions

Dissolution test

Both the United States Pharmacopoeia 2005 and the British Pharmacopoeia 2012 specifies that the amount of drug released (dissolution) should not be less than 80% of the labeled amount at 30 minutes. In the case of the ciprofloxacin tablets under study, the label claim is 500 mg which puts 80 % at 400 mg. At time zero (day 0), the ciprofloxacin tablets had an acceptable dissolution profile – releasing 89.12 % of the label claim after 30 mins. However, with increasing time there was a gradual reduction in the dissolution rate with the tablets exposed to all four storage conditions failing the test on day 44 where they had less than 80 % release of the label claim (see figures 3-6). The ciprofloxacin tablets exposed to UV light also failed the dissolution test from day 36 where they had less than 80 % release until day 44 of the evaluation.

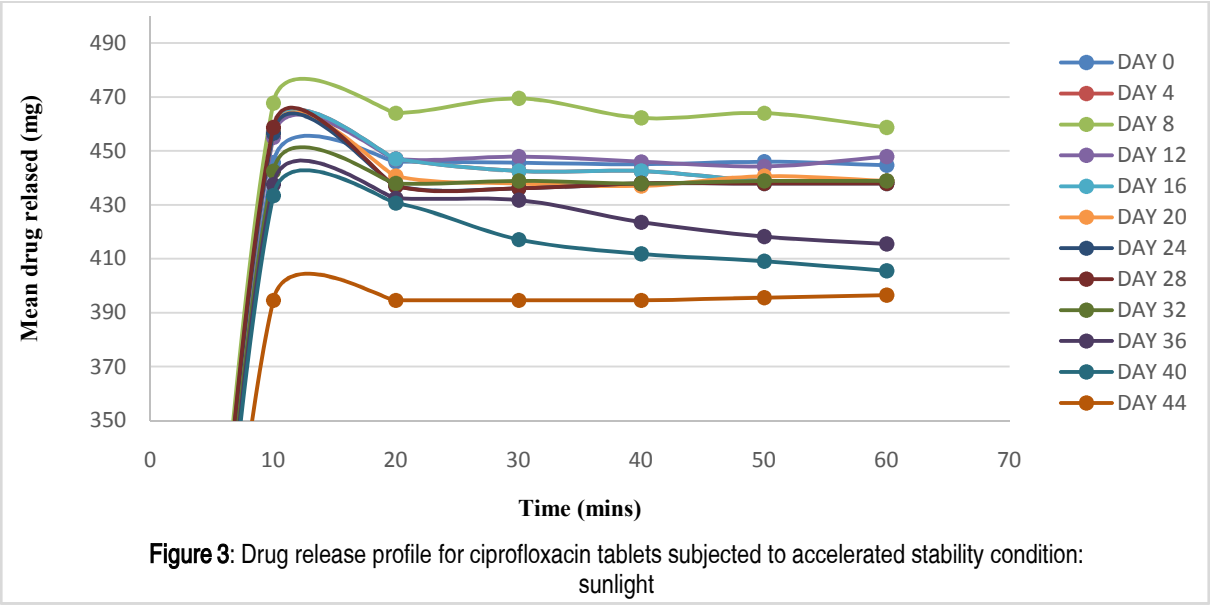

Figure 3: Drug release profile for ciprofloxacin tablets subjected to accelerated stability condition: sunlight

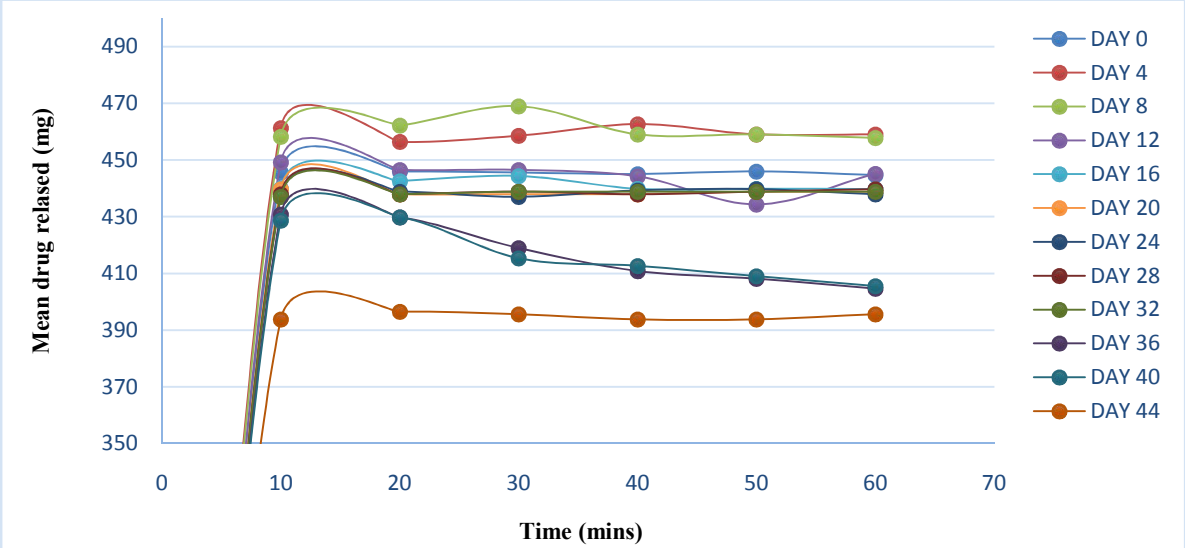

Figure 4: Drug release profile for ciprofloxacin tablets subjected to accelerated stability condition: moisture (75 % relative humidity)

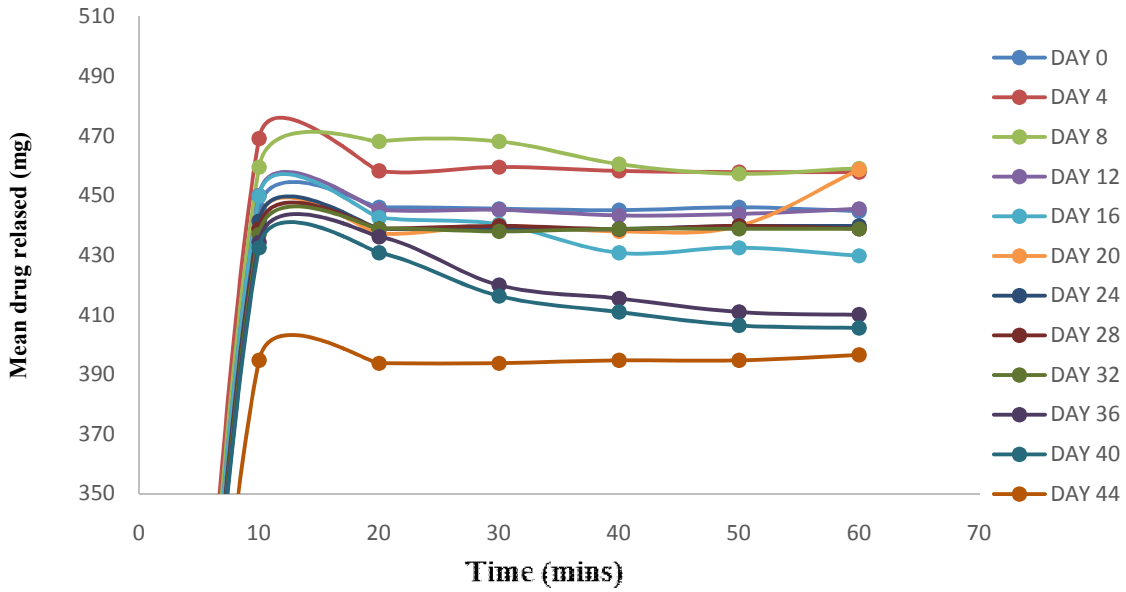

Figure 5: Drug release profile for ciprofloxacin tablets subjected to accelerated stability condition: temperature ± 40^{0}C

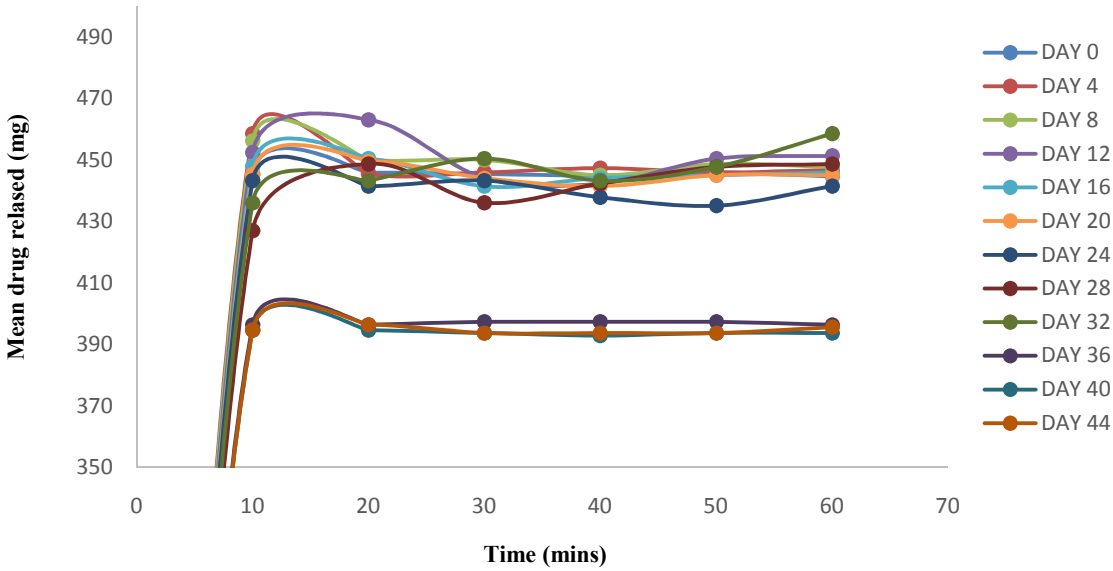

Figure 6: Drug release profile for ciprofloxacin tablets subjected to accelerated stability condition: uv light of 365nm for 4hours each day

Dissolution stability is considered a critical parameter not just from the standpoint of quality control but also for the impact on bioavailability of the product because significant changes of the in vitro release profile during storage in different conditions may affect its bioavailability. During aging, the absence of dissolution changes provides some assurance that the bioavailability remains intact [9]. The different excipients of a formulation may interact during exposure to high temperature or high humidity thereby reducing the in vitro dissolution. It is important to note that exposure of drugs which are susceptible, to light initiates many side reaction which

may affect the excipients interactions, surface of the formulation, drug uniformity and this may affect the dissolution rate profile and also bioavailability of the drugs [9].

This might explain why those tablets exposed to UV light failed the test, days ahead of their counterparts. The tablets exposed to UV light had a higher value for hardness than the others and show a slower dissolution rate at an earlier time (failing to comply with the specifications of 80 % drug release at 30 mins from day 36 of their exposure) than those exposed to the other three storage conditions (which failed to comply with the specifications of 80 % drug release at 30 mins on day 44 of their exposure) thus supporting the statement made earlier that, 'the higher the tablet hardness - the slower the drug dissolution rate.'

Content of active ingredient

The BP specification for content of ciprofloxacin tablet should be between 95-105 % [10]. Therefore, only those tablets exposed to UV light passed the test for all 44 days as they had a minimum of 96.83 % content on the 44th day. At day 0, all the tablets assayed passed this test, having a ciprofloxacin content of 99.43 %. However, those exposed to sunlight and temperature had content of ciprofloxacin hydrochloride below 95 % from day 4 which progressively declined to 78.36 % and 70.22 % respectively. For those exposed to moisture, they passed the test on days 4 - 8 and failed the test in subsequent days, having a ciprofloxacin content of 71.50 % on day 44. Generally, for all the storage conditions, there is a decrease in the content of ciprofloxacin hydrochloride after day 0, with those exposed to UV light being the least affected.

Conclusion and recommendation

Evaluation of the stability of ciprofloxacin tablets using such tablet parameters as hardness, disintegration, dissolution, weight uniformity and content of active ingredient tests after subjection to accelerated stability conditions showed considerable changes and deviations from the normal values obtained prior to exposure of the drugs to such stress conditions. For such parameters as hardness, disintegration and uniformity of weight, the tablets all remained within the specified ranges or complied with the specifications stated in the official books throughout the duration of study. As for the others- dissolution and content of active ingredient, the tablets failed the tests with increasing number of days under such stress conditions used in this study. Therefore, these changes noted in both the physical and chemical attributes of the ciprofloxacin tablets assayed indicates that they are not stable upon exposure to such accelerated storage conditions.

It is recommended that further studies be carried out to characterize the degradation products and determine their effect on therapeutic efficacy. Also, better approaches should be considered to improve the quality and stability of this medication especially with regards to packaging to ensure therapeutic efficacy especially in Nigeria where the environmental conditions are similar to those conditions used in this work.

Conflict of Interest

The authors declare no conflict of interest.

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