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A Comparative Photostability Study of Flagyrid, Metronidazole and Metrogyl Intravenous Infusion Brands Registered in Sudan

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ABSTRACT

Metronidazole 2-(2-methyl-5-nitro-1H-imidazole-1-yl) ethanol is a synthetic nitroimidazole derivative of antibacterial and antiprotozoal activity. It has different dosage forms. The stability of the Metronidazole intravenous of three different brands was studied in order to investigate the kinetics of photodegradation of this drug using a UV/ Vis spectroscopy. The degradation was carried out by the use of short (254 nm) and long UV (352 nm) lamps. The kinetics parameters of reaction order and the rate constants of the degradation were determined for the three brands products. The degradation process was found to follow first-order kinetics. The present study also reveals that the drug is light-sensitive. Thus, appropriate light protection is recommended during the storage and handling of metronidazole products. The study highlighted the fact that there is a correlation between the radiation intensity and degradation of metronidazole (photodegradation), i.e. as the radiation energy becomes stronger, the degradation rates increases. Furthermore, the rate of degradation was found to follow pseudo-first order reaction. It was also found that as the exposure time of these drugs to radiation increased the degradation was amplified. For all three brands were used in the investigation, the average rate of degradation when using long UV irradiation, and short UV radiation as stress factors were found to be,-0.0061 day^{-1} and -0.026 day^{-1} respectively. The obtained results show that UV/vis spectroscopy is satisfactory technique at the determination of metronidazole degradation kinetics in e intravenous products.

Keywords: UV/Vis spectroscopy, stability, kinetic of degradation, metronidazole.

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INTRODUCTION

The purpose of this photostability study is to determine the stability of the drug substance in the solid or dissolved state using either sunlight or much more light sources e.g. xenon arc Ultra Violet (UV)[1]. The stability of a pharmaceutical product should be investigated throughout the various stages of the development process. This process should start at the preformulation stage where the active drug substance is determined).. Stability and compatibility of the active pharmaceutical ingredient with various solvents, buffers, solutions, and excipents should be performed in order to reach the optimal formulation.

In general, for a drug substance, we need to investigate 3 types of stabilities-

1. Solid state stability drug only
2. Compatibility studies (The active pharmaceutical ingredient(API) and the excipients)
3. Solution phase stability

Photostability studies must be an integral part of the stress testing of pharmaceuticals in order to achieve the desired therapeutic effect and safety. Currently, these studies represent an important area of investigation because the photo degradation process can result in the loss result in loss of the potency of the drug an also in adverse effects due to the formation of minor toxic degradation products. This is especially important in the case of parenteral dosage forms, particularly antibacterial agents given by intravenous (I.V) route. The increased (use) of parenteral dosage forms in hospitals was verified in a survey revealing that. 40% of drugs dispensed to patients are injectable [2,3]. Such studies can explain several factors that affect the validity of drug products. These changes include chemical, biological and physical changes during the pre-clinical formulation stage, process development, packaging development and post- marketing life. The evaluation of physiochemical stability of a given product requires an understanding of the physical, biological and chemical properties of the drug substance[2-5]. The International Conference on Harmonization (ICH) guidelines presents the standard conditions for photostability studies and requires that stress testing must be carried out to elucidate the inherent stability characteristics of the active substance in a pharmaceutical preparation. Information about photostability of drugs can also help to determine the storage conditions of pharmaceutical products [1, 5]. The ICH Harmonized Tripartite Guideline on Stability Testing of New Drug Substances and Products recommends that a systematic approach to photostability should cover studies such as: (i) Tests on the drug substance. (ii) Tests on the exposed drug product outside of the immediate pack; and if necessary. (iii) Tests

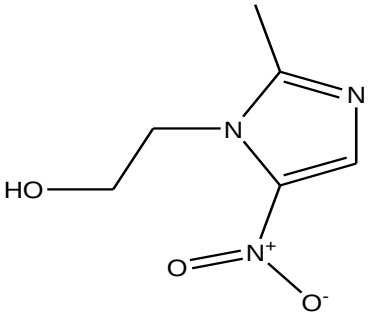
on the drug product in the immediate pack; and if necessary. (iv) Tests on the drug product in the marketing pack.

MATERIALS AND METHOD

Chemicals and Reagents

The chemicals and reagents used in this study are listed below

i. Metronidazole reference standard (Lab Tech Chemicals)



2- (2-methyl-5-nitro-1H-imidazole-1-yl) ethanol.

Figure 1: Metronidazole structure

ii. Hydrochloric acid AR (SDFCL fine chem. Limited, Mumbai).

iii. Distilled water, Daihan Lab Tech,

iv. Methanol (99.8%)(Lab Tech Chemicals),Belgium

Samples

Three different brands of Metromidazole for Intravenous Infusion were purchased from community pharmacies in Khartoum State. Their details are given below in Table 1.

Table1: Details of the Metronidazole different brands used in the study.

Brands	Manufacturer	Concentration	Manuf. Date	Expiry Date
Metronidazole(Brand A)	Jeddah, Saudi Arabia	500mg/100ml	12/2012	12/2015
Flagyrid,(Brand B)	Alpha-Aleppo,Syria	500mg/100ml	02/2013	02/2018
Metrogyl, (Brand C)	Unique pharmaceutical labs. India	500mg/100ml	01/2013	12/2015

Instruments

UV/vis Spectrophotometer model 1800 240 V, soft (Shimadzu corporation, Japan). pH meter 523, serial-No.19060060, Germany. UV lamps short (254nm) and long (365nm), upland CA91786, U.S.A. Weighing balance, serial-No, 1119291003, OHAUS, Swizterland

PREPARATION OF SAMPLES

Preparation of reference standard solutions

Standard solutions of metronidazole were prepared according to a validated and published method(6) .0.002g per 100ml was prepared by accurately weighing and dissolving 500mg of

standard metronidazole in a small quantity of methanol (99.8%)and diluted to 500ml with 0.1M HCl, then50 ml of the stock was further diluted to 500 ml with 0.1M HCl, 10 ml aliquots of this solution were transferred to a 50ml volumetric flask and the volume was completed to the mark by HCl solution. Five aliquots from the stock were prepared by taking 2.5, 5, 7.5, 10, 12.5 and 15ml from stock solution and completed in 100ml volumetric flask to produce 0.0005, 0.001, 0.0015, 0.002, 0.0025 and 0.003 g/100ml, respectively.

Preparation of reagent

8.8ml of concentrated HCl was transferred to a 1000 ml volumetric flask and the volume was completed using distilled water to prepared 0.1M HCl solution.

Preparation of drug samples

The amount (concentration) of Metronidazole infusion equivalent to 0.002 g/100ml was prepared using British pharmacopoeias (2012) official method (7) as follows**:** 10ml of Metronidazole infusion containing 50 mg was transferred to 100 ml volumetric flask, then the volume was completed with 0.1 M HCl. 10 ml of the resulting solution was diluted to 250 ml with 0.1 N HCl to produce a concentration of 0.002 mg/ 100ml. The absorbance was measured at the maximum at 277 nm, as recommended in the B.P and the content of $C_6H_9N_3O_3$ was calculated taking 375 as the value of $A^{1\%}_{1cm}$ at the maximum 277 nm.

Photostability

Samples were exposed to UV light (short and long wavelengths) for four days and measurements were taken after 24 hours from exposure to find the resultant concentration.

Data Analysis

Data analysis was performed by using Microsoft office Excel 2007.

RESULTS AND DISCUSSION

Cabilbration curve

The calibration curve data was obtained by using standard Metronidazole to assess the drug amount after being subjected to the stress conditions (see Table 2).

Table 2: The calibration curve data obtained by using standard Metronidazole

Conc.(mg/100ml)	Absorbance (in nm)
00.5	0.1785
1.0	0.357
1.5	0.5325
2.0	0.7531
2.5	0.8862
3	1.105

The plot of absorbance Vs concentration gave the curve shown in Figure 2.

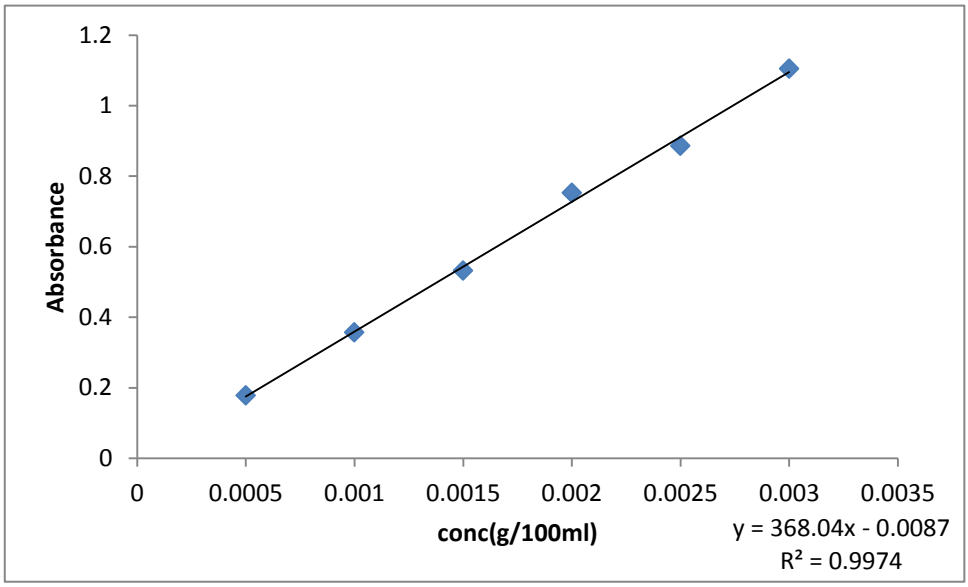


Figure 2: Calibration curve of standard solution of Metronidazole.

From the regression analysis, the correlation coefficient (R^2) was found to be 0.9974 which indicates strong correlation between concentration of standard and increase in absorbance.

Short UV lamp (254 nm) study

PH measurements

Three different brands of Metronidazole were irradiated by short UV lamp (254 nm) for four days and pH measurements showed that no significant change in the pH values before and after irradiation. The concentration at each day was measured using the calibration curve and the UV/vis instrument and the results are shown in Table 3.

Table 3: Quantitative determination of Metronidazole A in the samples that subjected to short UV lamp 254 nm for 4 days.

Time/day	$A_{277n\,m}$ (mean)	Conc (w/v)	Ln Conc	Content %(w/v)
0	0.7621	0.00203	-6.199	101.6%
1	0.7638	0.00204	-6.215	101.8%
2	0.7386	0.00196	-6.234	98.5%
3	0.7202	0.00192	-6.255	96.0%
4	0.7019	0.00187	-6.282	93.6%

Where: $A_{277n\,m}$: Absorbance at 277nm. Conc: Concentration

Drug A started to degrade when irradiated by short UV radiation which provides high energy. The concentration decreases from 101.6% to 93.6% after four day of contact. This value falls outside the British pharmacopoeia lower limit which is (95%). The plot of Ln C Vs time is shown in Fig 3.

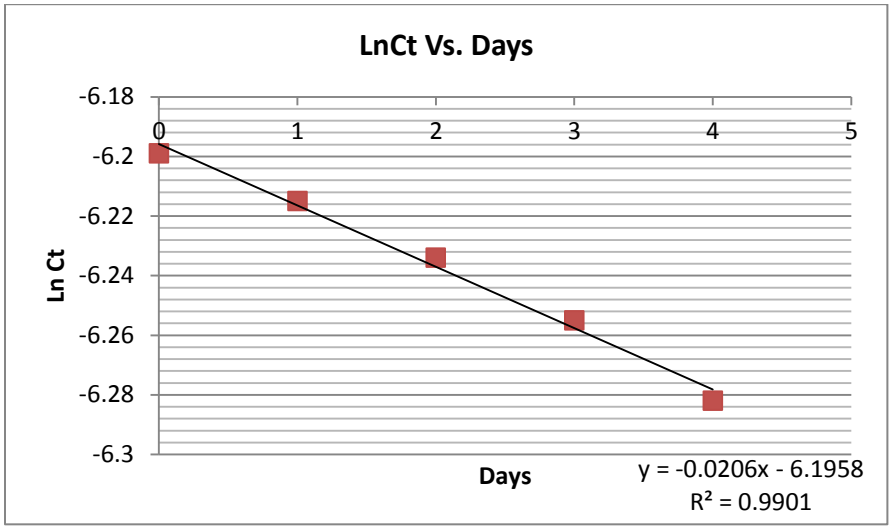


Figure 3: The plot of LnC_t Vs Days of drug A.

From the plot, it is clear that the degradation pattern follows first order reaction. Statistical analysis indicates that the correlation coefficient was found to be 0.99 which is considered as a strong correlation between exposure the time and the decrease in concentration. Using the following equation.

$\ln C_t = \ln C_0 - Kt$ .....(1)

The rate constant (K) was found to be K=-0.0206 day^{-1}

The half life was calculated using the following equation

$t_{1/2} = 0.693/K$ ..... (2)

From which $t_{1/2}$ was found to be =33.6 days.

The shelf life was obtained using the following equation:

$t_{10\%} = 2.303/K \log 100/90 = 0.105/k$

$t_{10\%} = 0.105/K$ ..... (3)

Where: $t_{10\%}$ is the half life time, $t_{0.9}$=0.105/0.0206 =5.1 days.

Brand B

Brand B was subjected to the same treatment and the results are tabulated below.

Table 4: Quantification of Metronidazole Brand B samples that subjected to short UV lamp (254 nm).

Time/day	$A_{277n\,m}$(mean)	Conc. (g/100ml)	Ln Conc.	Content %(w/v)
Zero	0.8062	0.00217	-6.13	108.5%
1	0.7921	0.00211	-6.16	105.5%
2	0.7687	0.00205	-6.19	102.1%
3	0.7513	0.00200	-6.21	100.5%
4	0.7239	0.00193	-6.25	96.5%

From results obtained in table 4 it was noticed that the drug degraded by short UV lamp and the effect start from day one until reached 96.5% at day four. Figure 4 explains the relation between concentration of brand B and time of irradiation.

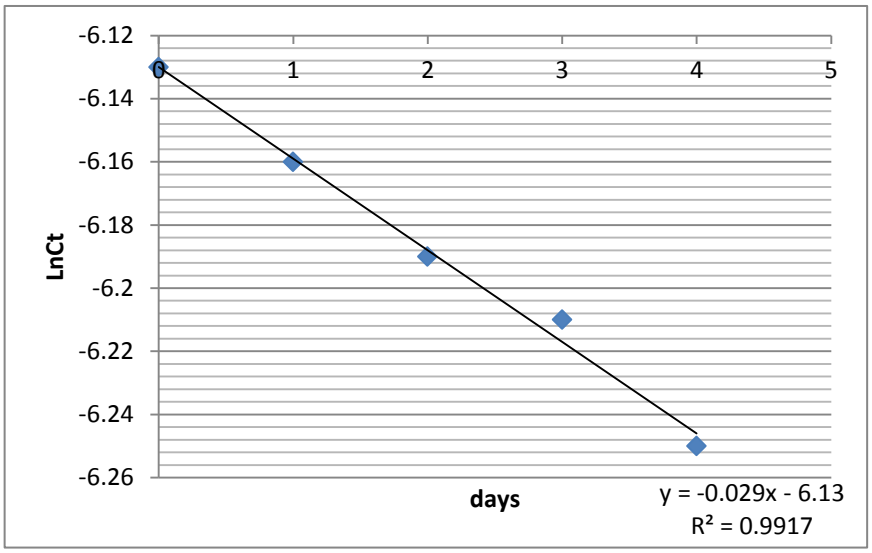


Figure 4: Showing plot of ln C_t Vs Days for drug B under short wavelength radiation.

A Form the graph, first order degradation is evident. The slope of this curves give the rate of degradation (Rate constant (K)) which was found to be = -0.029 day^{-1}

Using equation 2 and equation3, the shelf life and half life 23.9 3.6 days, respectively. were calculated as follows:

Brand C

Drug C was irradiated by short UV radiation (high energy) for four successive days and the results obtained are tabulated below.

Table 5: Quantification of Metronidazole Brand C in the samples that being subjected to Short UV radiation (254 nm) for four days.

Time/day	A_{277nm}(mean)	Conc. (g/100ml)	Ln Conc.	Content %
Zero	0.8001	0.00213	-6.15	106.5%
1	0.7764	0.00207	-6.18	103.5%
2	0.7501	0.00200	-6.21	100.5%
3	0.7351	0.00196	-6.23	98.0%
4	0.7087	0.00189	-6.27	94.6%

It was noticed that drug was affected by short UV radiation and the concentration was dropped to 94.6% (original value was 106.5%) The value of 95% is the lower limit which is set in the British Pharmacopeia(2012). The plot of concentration of drug C Vs time of exposure is shown below in figure 5.

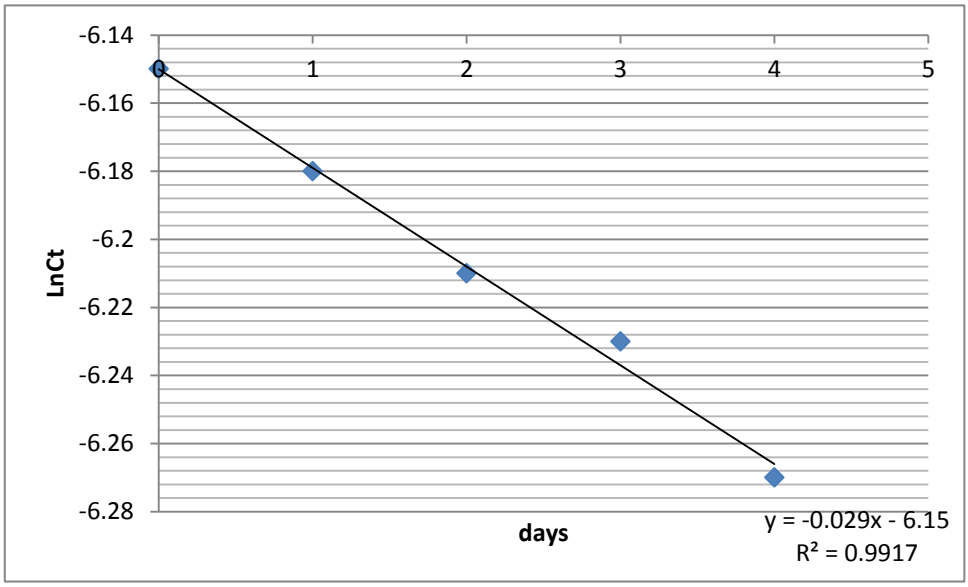


Figure 5: Showing plot of ln Ct Vs days for drug C under short UV irradiation.

From the graph, the rate constant was found to be -0.029 day^{-1} and the half life and shelf life are calculated are 23.9 days and 36 days, respectively. PH measurements of the three brands before and after irradiation showed no significance difference. A summary of the kinetics data obtained are given in Table 6.

Table 6: Degradation Rate constants, half lives and shelf lives of 3 Brands Metronidazole intravenous infusions, after exposed to short UV (254 nm) radiation for 4 days.

Sample	Rate constant K (day^{-1})	$T_{1/2}$ (Rounded/days)	$T_{0.9}$ (Rounded/days)
Drug A	-0.0206	34	5
Drug B	-0.029	24	4
Drug C	-0.029	24	4

From the above table , a one can conclude that brand B and C have identical rate constant, half life and shelf life whereas brand A has a lower rate constant and hence longer half life and shelf life.

Kinetic study of the effect of long UV lamp (365nm) on the three Brands.

The three Brands of Metronidazole infusion were subjected to long UV radiation for four successive days and the absorbance was measured every day to determine the concentration of the active pharmaceutical ingredient and the results obtained are tabulated below.

Brand A

Concentration measurements of Brand Aafter irradiation with long UV lamp (365nm) are shown in Table 7.

Table 7: Quantification of Metronidazole Brand A in the samples that being subjected to long UV lamp (365 nm) for four days.

Time/days	$A_{277n\,m}$ (mean)	Conc. (g/100ml)	Ln Conc.	Content %(w/v)
Zero	0.738	0.00196	-6.234	98.0%
1	0.7317	0.00195	-6.239	97.5%
2	0.7291	0.00194	-6.245	97.2%
3	0.7237	0.00193	-6.250	96.5%
4	0.7212	0.00192	-6.255	96.2%

The relationship between concentration and time of exposure for drug A under long UV irradiation is shown in figure 6.

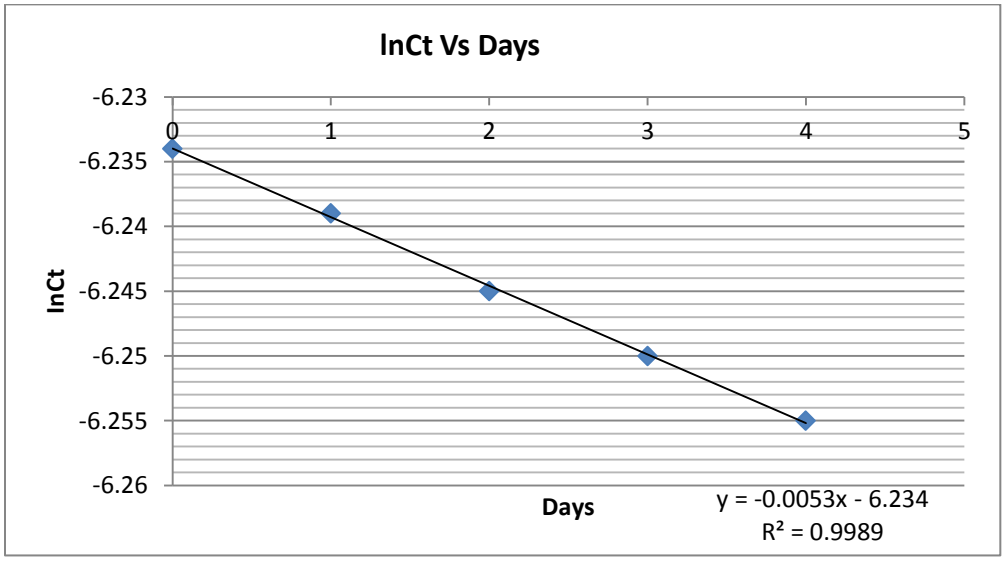


Figure 6: Showing Plot of concentration Vs. Time for Drug A under long UV irradiation.

The degradation rate, and shelf life and half life were found to be, K= -0.0053 day^{-1},$t_{0.5}$= 0.693/0.0053=130.8 days., $t_{0.9}$= 0.105/0.0053=19.2 days.

Brand B

After treating brand B with long UV lamp (365nm), the concentration was measured and the obtained results are tabulated in Table 8.

Table 8: Quantification of Metronidazole Brand B in the samples that being subjected to long UV lamp (365 nm) for 4 days.

Time/days	$A_{277n\,m}$ (mean)	Conc. (g/100ml)	Ln Conc.	Content %(w/v)
Zero	0.7392	0.00197	-6.229	98.5%
1	0.7341	0.00196	-6.236	98.0%
2	0.7305	0.00195	-6.241	97.5%
3	0.7254	0.00193	-6.248	96.5%
4	0.7203	0.00192	-6.255	96.0%

From results in the above table, it was noticed that the concentration of API had decreased with prolonged irradiation with long UV lamp. In spite of the fact that the energy content of the long

UV irradiation is relatively lower than the shorter counterpart, it was enough to cause photo degradation.

Figure 7 shows the plot of ln concentration Vs time for drug B under long UV radiation.

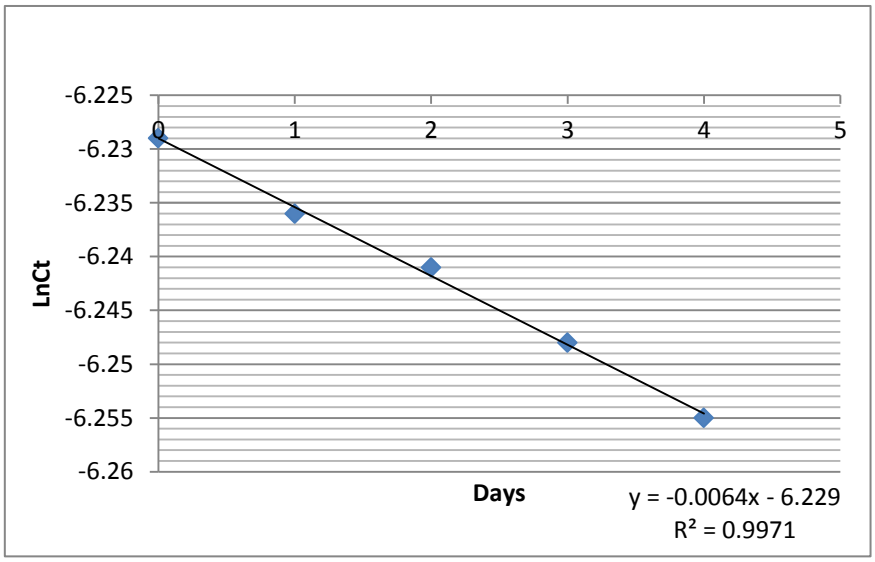


Figure 7: showing the plot of Ln C_t Vs time/days for drug B under long UV radiation.

Form the graph, the correlation coefficient R^2 was found to be 0.9971 (strong correlation), and the rate of degradation was determined from the equation, then shelf life and half life were calculated and the results were.

K = -0.0064 day^{-1}

$t_{0.5}$ = 0.693/0.0064 = 108.3days

$t_{0.9}$ = 0.105/0.0064 = 16.4 days

Brand C and after being treated in a similar manner, its concentration was measured and the results are shown in Table 10.

Brand C

The results of drug C is given in table 9.

Table 9: Quantification of Metronidazole Brand C that subjected to long UV radiation (365 nm).

Time/day	A_{277nm} (mean)	Conc. (g/100ml)	Ln Conc.	Content %
Zero	0.8003	0.00213	-6.15	106.5%
1	0.7764	0.00207	-6.18	103.5%
2	0.7501	0.00200	-6.21	100.5%
3	0.7351	0.00196	-6.24	98.0%
4	0.7087	0.00189	-6.26	94.6%

Figure 8 explains the relation between concentration and time of drug C after 4 days of long UV irradiated.

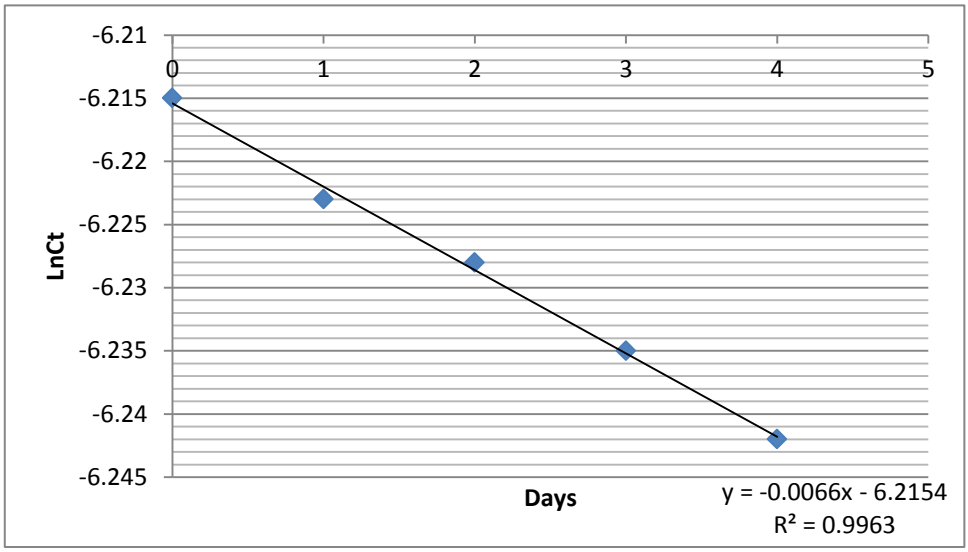


Figure 8**: The relation between concentration and time of drug C after 4 days of long UV exposed.**

The rate of degradation, half life and the shelf life were found to be -0.0066 day^{-1}. 105 days and 15.9 days, respectively.

Three brands were compared and the results are shown in Table 10.

Table 10: Degradation Rate constants, half lives and shelf lives of 3 Brands Metronidazole intravenous infusions, after exposed to long UV (365 nm) radiation for 4 days.

Sample	K	$T_{1/2}$ (Rounded/days)	$T_{0.9}$ (Rounded/days)
Drug A	-0.0053	131	19
Drug B	-0.0064	108	16
Drug C	-0.0066	105	16

The rate of degradation of drug A was -0.0053, for drug B was -0.0064 which is relatively higher than A, and was -0.0066 for drug C which is highest one that's indicated drug A is the most stable brand followed by brand B and finally brand C. In some cases, light exposure can induce chemical degradation in the active pharmaceutical ingredient (API). However, other forms of electromagnetic radiation can cause photo degradation of drug substances and products. The most problematic ambient light wavelengths are the long ultra violet and short visible (blue) lights, partially because of the energy content (high energy wave lengths)[5].

Light effect increases as the wavelength of the radiation source becomes shorter and the exposure time increases.

- Many drugs fade or darken on exposure to light and this leads to an aesthetic problem which can be controlled by using

1 Amber Glass Container

2 Opaque Container

3 Incorporating a Dye

CONCLUSION

From the investigation of the different brands of Metronidazole which were subjected to long and short wavelength radiation, the following conclusions could be drawn. All the drugs complied with the limits stated in the B.P (2012) before irradiation. All the brands were affected by short and long wavelength radiation to different extents and according to the energy content of radiation source. The rate of degradation of Metronidazole solution was found to follow pseudo-first order reaction. UV/vis spectrophotometry is an excellent technique to follow the degradation of Metronidazole infusion due to its simplicity and its ability to perform quantitative measurements.

REFERENCES

1. International Conference on Harmonization (ICH) (1995), Guideline for the Photostability Testing of New Drug Substances and Products. Step 2. (ICH Topic Q 1 B).
2. Baltezor. M. Stability Testing of drug products. In Grimm W (ed), Paperback A. P, Wissenchaftliche Verlagsg Fesellschaft . Stuttgart, 1985; 16: 27.
3. H. Hjorth Tonnesen (Ed.), Photostability of Drugs and Drug Formulations, Taylor & Francis, London .1996; 287-304 ; b: 83-110 .
4. Cartensen J.T . Drug Stability: Principles and practices. Marcel Dekker, Inc., 1990; 43: 45.
5. ICH Harmonized tripartite guidelines for Stability Testing of new drug substances and products – Q1A (R2).
6. Amit Alexander1, Rashmi Chaurasia, Junaid Khan, Swarna, Sanjeev Sahu, Sandip Patel. Spectrophotometric Method of Standard Curve preparation and calculation for Metronidazole. 2011;2 (1)
7. British pharmacopoeia, The BP Commission, Vol. iii, Her Majesty Stationary Office (HMSO) London, U.K Dec 2012: 3065.