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## EXTENT OF PHOTO-DEGRADATION OF THREE DIFFERENT DIAZEPAM TABLET FORMULATIONS AVAILABLE IN TRANSPARENT PACKAGING: AN UV ANALYSIS

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### Keywords:

Photolytic degradation, UV Spectroscopy, Diazepam, Light-Protective

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**ABSTRACT:** This study was designed to determine the extent of photolytic degradation of diazepam tablets, without light-protective packaging, available in the market. The UV spectroscopy method was designed and applied for this purpose. A total of 2160 tablets (each tablet contained 5 mg diazepam) of three different brand formulations A, B and C were chosen randomly. These tablets were then exposed to different indirect and direct lighting conditions i.e. normal room light condition (for 60 days), direct sunlight exposure (6 hours per day for 3 days), 25 Watt incandescent light bulb exposure and 40 Watt incandescent light bulb exposure (6 hours per day for 3 days). Samples from these light exposures were analyzed for potency determination using UV spectrophotometer and compared against the tablets kept in dark place (Control). Tablets of all three formulations showed marked degradation. The overall average degradations were  $23.64\% \pm 5.53\%$ ;  $28.36\% \pm 4.87\%$ ;  $19.72\% \pm 4.83\%$  and  $25.40\% \pm 4.17\%$  respectively for normal room light, direct sunlight, 25 Watt and 40 Watt incandescent bulbs whereas tablets kept in dark place showed little or no decrease in potency ( $0.01\% \pm 0.01\%$ ). These results suggest that the diazepam tablets should be marketed in light-protected opaque packaging to retain their potency and optimum therapeutic effect. At the same time, the existing transparent packaging should also be replaced with the opaque ones by the manufacturers.

**INTRODUCTION:** With the advancement of modern civilization, complexity of human nature has also been increased. Complicated emotional states are being more and more common among people. Anxiety is one of those complex psychological conditions. Anxiety, according to American Psychological Association (APA)<sup>1</sup>, is an emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure.

Other symptoms include restlessness, irritability, muscle tension, excessive tension about the worst outcome of any situation, increased urination rate and so on<sup>2</sup>. Anxiolytic drugs, commonly known as sedatives are the class of drugs which can reduce the symptoms associated with anxiety and induce calmness in an individual, generally working on the central nervous system<sup>3</sup>. There are many drugs, which are used therapeutically as anxiolytic drugs. Among them benzodiazepines is a class of wide recognition and are being used for a long time in the medical field.

Diazepam is an anxiolytic drug of benzodiazepine group which was the first member of this class. It has a very low polarity hence it is rapidly absorbed after oral administration. Diazepam has the similar mechanism of action like all other benzodiazepines.

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Gamma amino butyric acid (GABA) influences the excitement of the brain by the binding to the GABA receptor. Upon binding it accelerates the chloride influx into the cell to cause hyperpolarization. This hyper-polarization reduces excitation of the CNS. Diazepam increases the GABA<sub>α</sub> receptor activity in the brain. They bind to the GABA<sub>γ</sub> receptors to produce positive allosteric change of the GABA<sub>α</sub> receptors. This positive change increases the activity of those receptors as well as the influx of chloride ions. Thus, diazepam decreases the excitation of the CNS as well as anxiety<sup>4,5,6</sup>.

Photolytic degradation refers to the breakdown or alteration of chemical structure due to the influence of light radiation. In other words, photolysis means the reduction in potency of any compound in presence of light. The rate of photolytic degradation depends on the intensity of the incident light as well as the extent of light absorbed by the compound<sup>7</sup>. Photolytic degradation may be oxidative or non-oxidative. Non-oxidative photolysis accelerates isomerization, dimerization, cyclization, rearrangement etc. On the other hand, oxidative photolysis produces oxides or peroxides<sup>8</sup>. The wavelength range of 300-800nm is the most agreeable range for photolysis<sup>9,10</sup>.

Diazepam is available in pharmaceutical market in a number of dosage forms including oral tablet, oral capsules, oral concentrated solutions, IV suspension, rectal kit etc<sup>11</sup>. However, tablet dosage form is most common for the administration of diazepam. Diazepam has been reported to have photosensitivity, and this photo-degradation is more obvious in aquatic environment<sup>12,13</sup>.

However, many of the tablets marketed in Bangladesh are uncoated as well as are packaged in transparent normal blister packaging. Since, they are neither packaged in an opaque packaging nor coated to prevent light access, there is an obvious possibility of degradation of diazepam by light.

The aim of the present study was to evaluate the extent of potency reduction of diazepam tablets which are not light protected by packaging or tablet coating. Three different formulations were chosen to unearth the degradation extent.

## MATERIALS AND METHODS:

A total of 2250 tablets (750 tablets of each brand formulation) were purchased from a local pharmacy store in Dhaka, Bangladesh for this study. All tablets of each brand formulation were of the same batch and available in blister packs which were not light protective. From here a total of 2160 (720 tablets from each brand formulation) tablets were used for the experiments.

### Instruments:

Philips Incandescent Light bulb, 25 Watt, Bangladesh; Philips Incandescent Light bulb, 40 Watt, Bangladesh; 12 inch lab thermometer, Midwest Home brewing and Winemaking Supplies, USA; Shimadzu UV 1800, Japan; Distill Water plant, Bibby Scientific W 4000, UK; Electronic balance Shimadzy AY 220, Japan.

### Determination of Lamda Max ( $\lambda_{max}$ ):

UV lamda max ( $\lambda_{max}$ ) was determined (240.1 nm) in 0.1N sulfuric acid solution (made freshly from 98% w/v stock solution) using the Diazepam reference standard (99.02% potency) of R L fine Chem (RLFC), India.

### Preparation of Standard Curve:

A total of 9 concentrations (0.001-0.009 mg/ml) of diazepam were prepared using the 0.1N H<sub>2</sub>SO<sub>4</sub> solution and the absorbances were measured at the  $\lambda_{max}$  240.1 nm. Then plotting these absorbances against their respective concentrations yielded a Standard Curve with the following equation:

$$Y=89.68x+0.017; R^2=0.997$$

Where, Y = Absorbance (Abs) X = Concentration of the drug (mg/ml)

This equation was used to determine the drug formulation potencies in this study. The tablets were exposed to the following lighting conditions keeping inside their blister packs.

### Normal Room Light Exposure:

The duration of this experiment was for two months. From Formulation-A, 65 tablets were kept under normal room light condition (Indirect light exposure) and 25 tablets were kept away from light in a dark place as Control. From here, initially 10

tablets were analyzed by UV spectroscopy. It included 5 tablets from controls and 5 tablets from the samples. Then at every 15 days intervals 15 tablets from light exposure (Sample 1A, Sample 2 A, Sample 3 A) and 5 tablets from the Control were taken for analysis. Each of Sample 1A, Sample 1B and Sample 1C consisted of 5 tablets.

Same procedures for sampling and analysis were followed for Formulation B (Sample 1 B, Sample 2 B, Sample 3B and Control) and Formulation C (Sample 1 C, Sample 2 C, Sample 3C and Control).

### Direct Sunlight Exposure:

From Formulation A, 50 tablets were exposed to direct sunlight in a hot summer day in April, 2015 on the roof of East west University Campus, Aftabnagar, Dhaka, Bangladesh. 20 tablets were kept in dark as Control. Initially 10 tablets (5 Controls + 5 samples before light exposure) were analyzed. After that every 2 hour intervals 20 tablets were taken for UV analysis up to 6 hours. In every interval, these 20 tablets included 5 tablets from Controls and 15 tablets from Samples (Sample 1A, 2A and 3A) exposed to light. Each of these 1A, 2A and 3A samples consisted of 5 tablets.

This experiment was repeated twice (Control 2A with Samples 4A, 5A and 6A; and Control 3A with Samples 7A, 8A and 9A) in two different days with

the same weather to check the reproducibility of the results.

The same procedure was followed for Formulation B (Control 1B with Samples 1B, 2B and 3B; Control 2B with Samples 4B, 5B and 6B; and Control 3B with Samples 7B, 8B and 9B) and Formulation C (Control 3C with Samples 1C, 2C and 3C; Control 2C with Sample 4C, 5C and 6C; and Control 3C with Sample 7C, 8C and 9C).

**Exposure to 25Watt and 40Watt Incandescent Light bulbs:** Same sampling and analytical procedure was also followed for two different direct lighting conditions i.e. exposure to 25Watt and 40Watt incandescent light bulbs.

**Analytical Procedure:** For Controls and Samples, each analytical reading involved 5 tablets. These were crushed in a mortar. Then the weight equivalent to the average weight of a single respective formulation tablet was taken. This weighed crushed tablet powder was then diluted 1000 times into 0.1N sulfuric acid solution for the UV analysis of potency. The absorbances found were used in the Standard Curve Equation to calculate the potencies.

### RESULTS AND DISCUSSION:

All the results from this study are shown in the tables and figures as follows:

**TABLE 1: EXTENT OF PHOTOLYTIC DEGRADATION OF FORMULATION A, B AND C UNDER NORMAL ROOM-LIGHT**

Test type	Initial Potency (%)	Potency after 60 days (%)	Potency Decrease (%)	Mean Potency decrease (%) of Each Formulation	Standard deviation (+/-) of Each Formulation (%)	Mean Potency decrease (%)	Standard deviation +/- (%)
Sample 1 A	98.90	79.80	19.31	20.87	1.88	23.64	5.53
Sample 2 A	98.91	78.80	20.33				
Sample 3 A	98.90	76.20	22.95				
Sample 1 B	99.00	81.40	17.78	19.26	1.43		
Sample 2 B	99.00	79.80	19.39				
Sample 3 B	99.02	78.60	20.62				
Sample 1 C	96.00	66.88	30.33	30.78	0.38		
Sample 2 C	95.99	66.24	30.99				
Sample 3 C	96.00	66.24	31.00				

**TABLE 2: EXTENT OF PHOTOLYTIC DEGRADATION OF FORMULATION A, B AND C UNDER DIRECT SUNLIGHT**

Formulation	Test type	Initial Potency (%)	Potency after 6 hours (%)	Potency Decrease (%)	Mean Potency decrease (%) of Each Formulation	Standard deviation (+/-) of Each Formulation (%)	Mean Potency decrease (%)	Standard deviation +/- (%)
<b>A</b>	Sample 1A	98.80	77.60	21.46	25.79	4.05	28.36	4.87
	Sample 2A	98.79	78.00	21.04				
	Sample 3A	98.80	76.80	22.27				
	Sample 4A	98.61	74.40	24.55				
	Sample 5A	98.61	73.80	25.16				
	Sample 6A	98.63	73.20	25.78				
	Sample 7A	98.78	68.40	30.76				
	Sample 8A	98.78	70.20	28.93				
	Sample 9A	98.79	67.00	32.18				
<b>B</b>	Sample 1B	98.80	77.60	21.46	25.84	4.11		
	Sample 2B	98.80	78.00	21.05				
	Sample 3B	98.81	76.80	22.28				
	Sample 4B	98.60	74.40	24.54				
	Sample 5B	98.60	73.80	25.15				
	Sample 6B	98.60	73.20	25.76				
	Sample 7B	98.99	68.40	30.90				
	Sample 8B	99.00	70.20	29.09				
	Sample 9B	99.00	67.00	32.32				
<b>C</b>	Sample 1C	95.84	64.03	33.19	33.44	0.22		
	Sample 2C	95.81	63.78	33.43				
	Sample 3C	95.83	63.63	33.60				
	Sample 4C	96.01	63.78	33.57				
	Sample 5C	96.00	63.78	33.56				
	Sample 6C	96.01	63.53	33.83				
	Sample 7C	95.78	64.05	33.13				
	Sample 8C	95.79	63.88	33.31				
	Sample 9C	95.79	63.83	33.36				

**TABLE 3: EXTENT OF PHOTOLYTIC DEGRADATION OF FORMULATION A, B AND C UNDER 25 WATT INCANDESCENT LIGHT**

Formulation	Test type	Initial Potency (%)	Potency after 6 hours (%)	Potency Decrease (%)	Mean Potency decrease (%) of Each Formulation	Standard deviation (+/-) of Each Formulation (%)	Mean Potency decrease (%)	Standard deviation +/- (%)
<b>A</b>	Sample 1A	98.89	87.40	11.62	15.64	1.90	19.72	4.83
	Sample 2A	98.87	84.60	14.43				
	Sample 3A	98.89	83.80	15.26				
	Sample 4A	98.61	83.80	15.02				
	Sample 5A	98.62	82.80	16.04				
	Sample 6A	98.62	81.20	17.66				
	Sample 7A	98.93	83.00	16.10				
	Sample 8A	98.94	82.40	16.72				
	Sample 9A	98.93	81.20	17.92				
<b>B</b>	Sample 1B	99.00	81.60	17.58	17.40	0.97		
	Sample 2B	99.01	82.60	16.57				
	Sample 3B	99.00	81.20	17.98				
	Sample 4B	98.91	82.80	16.29				
	Sample 5B	98.90	82.20	16.89				
	Sample 6B	98.92	81.40	17.71				
	Sample 7B	99.12	82.60	16.67				
	Sample 8B	99.12	81.80	17.47				
	Sample 9B	99.10	79.80	19.48				

<b>C</b>	Sample 1C	95.79	70.92	25.96	26.12	0.71
	Sample 2C	95.76	70.73	26.14		
	Sample 3C	95.80	70.03	26.90		
	Sample 4C	95.50	72.17	24.43		
	Sample 5C	95.48	70.79	25.86		
	Sample 6C	95.48	70.30	26.37		
	Sample 7C	95.46	70.13	26.53		
	Sample 8C	95.47	70.13	26.54		
	Sample 9C	95.46	70.30	26.36		

**TABLE 4: EXTENT OF PHOTOLYTIC DEGRADATION OF FORMULATION A, B AND C UNDER 40 WATT INCANDESCENT LIGHT**

Formulation	Test type	Initial Potency (%)	Potency after 6 hours (%)	Potency Decrease (%)	Mean Potency decrease (%) of Each Formulation	Standard deviation (+/-) of Each Formulation (%)	Mean Potency decrease (%)	Standard deviation +/- (%)
<b>A</b>	Sample 1A	98.82	74.40	24.71	25.18	2.34	25.40	4.17
	Sample 2A	98.82	73.80	25.32				
	Sample 3A	98.84	73.20	25.94				
	Sample 4A	98.89	78.20	20.92				
	Sample 5A	98.89	76.40	22.74				
	Sample 6A	98.88	74.20	24.96				
	Sample 7A	98.73	73.20	25.86				
	Sample 8A	98.73	71.80	27.28				
	Sample 9A	98.74	70.20	28.90				
<b>B</b>	Sample 1B	98.40	79.80	18.90	21.52	3.92		
	Sample 2B	98.44	82.60	16.09				
	Sample 3B	98.44	81.20	17.51				
	Sample 4B	98.60	78.60	20.28				
	Sample 5B	98.63	78.00	20.92				
	Sample 6B	98.60	76.80	22.11				
	Sample 7B	98.31	74.20	24.52				
	Sample 8B	98.34	73.80	24.95				
	Sample 9B	98.33	70.40	28.40				
<b>C</b>	Sample 1C	95.99	67.55	29.63	29.49	0.15		
	Sample 2C	95.98	67.55	29.62				
	Sample 3C	95.98	67.46	29.71				
	Sample 4C	95.97	67.76	29.39				
	Sample 5C	95.98	67.76	29.40				
	Sample 6C	95.97	67.57	29.59				
	Sample 7C	95.78	67.73	29.29				
	Sample 8C	95.79	67.71	29.31				
	Sample 9C	95.78	67.59	29.43				

**TABLE 5: EXTENT OF PHOTOLYTIC DEGRADATION OF FORMULATION A, B AND C CONTROLS**

Formulation	Test type	Control	Initial Potency(%)	Potency at the end of study (%)	Potency Decrease (%)	Mean Potency decrease (%)	Standard deviation +/- (%)
<b>A</b>	25 Watt Bulb Light	Control 1	98.89	98.88	0.01	<b>0.01</b>	<b>0.01</b>
		Control 2	98.64	98.64	0.00		
		Control 3	98.93	98.91	0.02		
	40 Watt Bulb Light	Control 1	98.82	98.83	-0.01		
		Control 2	98.89	98.88	0.01		
		Control 3	98.73	98.73	0.00		
	Direct Sunlight	Control 1	98.80	98.80	0.00		
		Control 2	98.61	98.59	0.02		
		Control 3	98.79	98.79	0.00		



<b>B</b>	25 Watt Bulb Light	Control 1	99.00	99.01	-0.01
		Control 2	98.91	98.91	0.00
		Control 3	99.12	99.11	0.01
	40 Watt Bulb Light	Control 1	98.40	98.39	0.01
		Control 2	98.60	98.60	0.00
		Control 3	98.31	98.28	0.03
	Direct Sunlight	Control 1	98.80	98.80	0.00
		Control 2	98.60	98.60	0.00
		Control 3	99.00	99.00	0.00
<b>C</b>	25 Watt Bulb Light	Control 1	95.79	95.79	0.00
		Control 2	95.48	95.47	0.01
		Control 3	95.46	95.46	0.00
	40 Watt Bulb Light	Control 1	95.98	95.98	0.00
		Control 2	95.97	95.95	0.02
		Control 3	95.78	95.78	0.00
	Direct Sunlight	Control 1	95.81	95.81	0.00
		Control 2	96.01	96.01	0.00
		Control 3	95.78	95.75	0.03

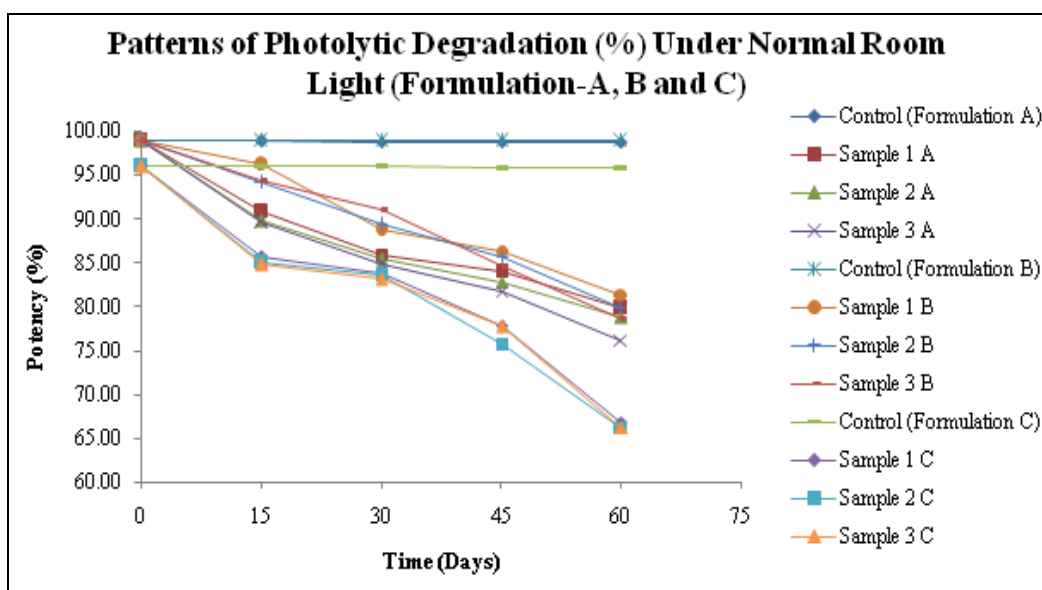


FIG. 1: PATTERN OF PHOTO-DEGRADATION OF ALL FORMULATIONS UNDER NORMAL ROOM LIGHT CONDITIONS

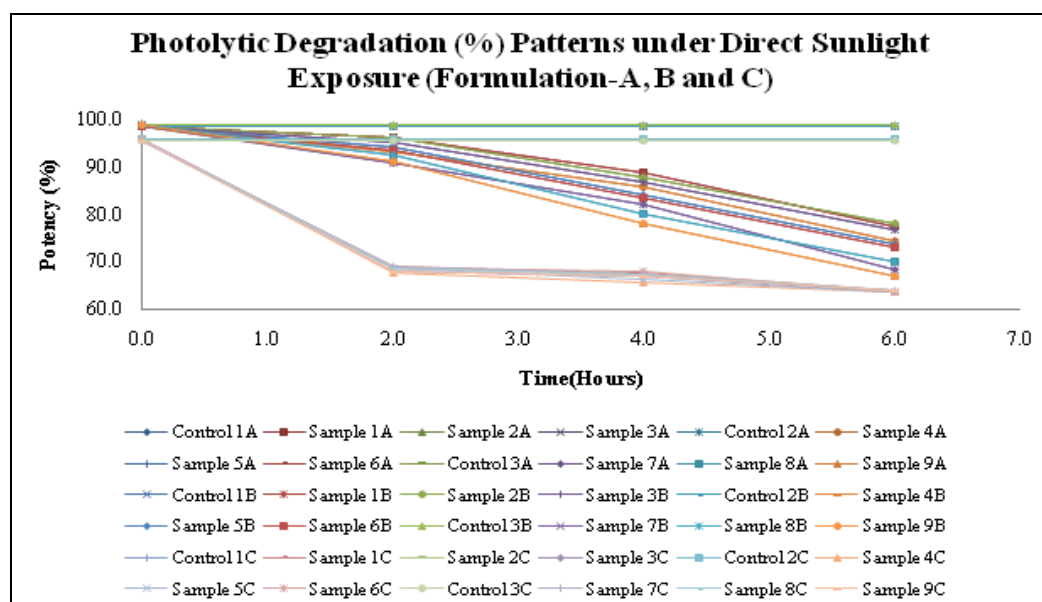


FIG. 2: PATTERN OF PHOTO-DEGRADATION OF ALL FORMULATIONS UNDER DIRECT SUNLIGHT

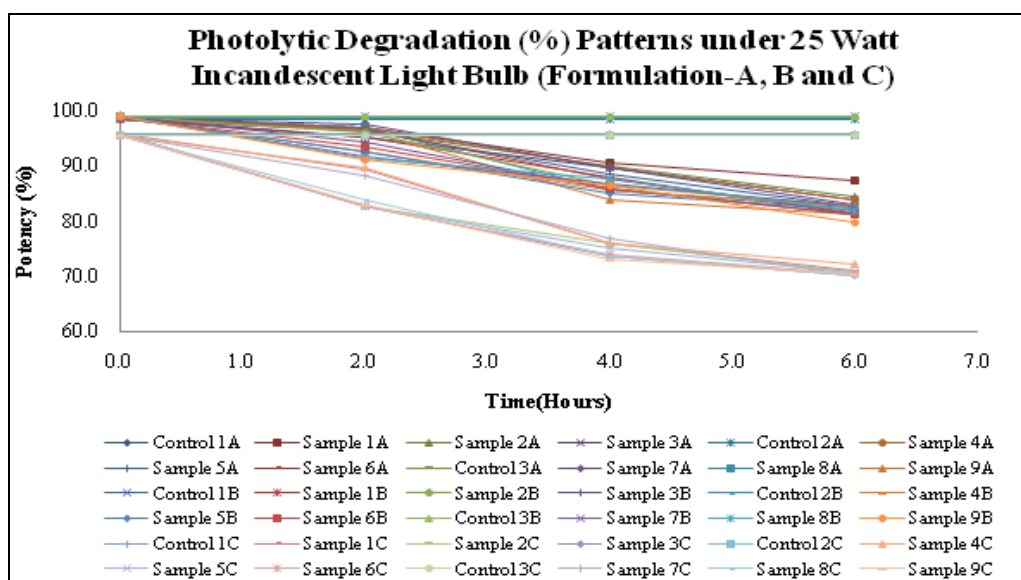


FIG. 3: PATTERN OF PHOTO-DEGRADATION OF ALL FORMULATIONS UNDER 25 WATT INCANDESCENT LIGHT

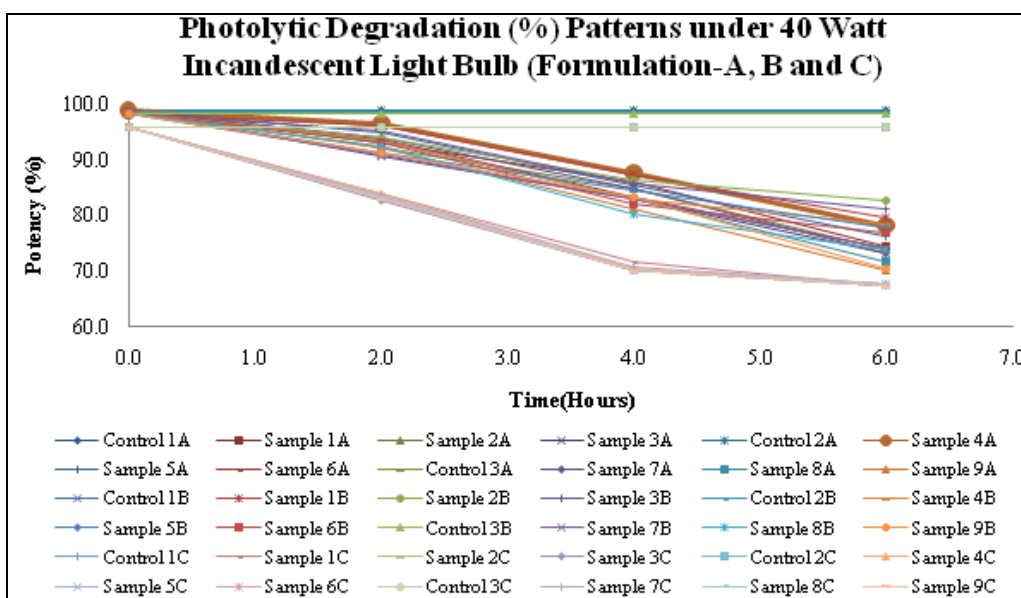


FIG. 4: PATTERN OF PHOTO-DEGRADATION OF ALL FORMULATIONS UNDER 40 WATT INCANDESCENT LIGHT

Our research program was aimed to evaluate the extent of photolytic degradation of randomly selected three formulations of diazepam, which were not available in opaque packaging. As mentioned before, all the formulations were exposed to four different lighting conditions (Normal room light, direct sunlight, 25 watt bulb and 40 watt bulb). All of the formulations experienced gradual decrease in potency (Fig. 1,2,3 and 4), although the extent of reduction in potency was different for various lighting conditions as well as for three different formulations. Unexposed tablets which were labeled as ‘Controls’ experienced no detectable reduction in potency and displayed consistent potency level within the time

frame of our research. i.e. 0.01% potency decrease with the standard deviation of 0.01% (Table 5).

Under normal room light condition (Table 1 and Fig. 1), all the formulation showed decrease in potency. The mean potency decrease was 23.64% with a standard deviation of 5.53% for all the formulations. However, Formulation C displayed the greatest extent in potency reduction (30.78%±0.38%). On the other hand, Formulation B displayed least potency diminution (19.26%±1.43%). This can be attributed to their formulation variables including the quality of API and excipients as well as their manufacturing parameters like moisture content, degree of mixing

etc.<sup>14, 15, 16</sup>. Presence of any excipient that can accelerate free radical reaction can also be the cause of photo-degradation resulting greater potency reduction<sup>17, 18</sup>. The purity of active pharmaceutical ingredient (API) can also contribute to different patterns of potency reduction in different formulations<sup>19</sup>. Rare, but not unusual, potency can also be dependent on the thickness and polymer quality of blister packaging because the photolytic degradation directly depends on the intensity of the radiation<sup>7</sup>.

Direct sunlight exposure (**Table 2, Fig. 2**) to all the formulations taken for the research program was carried out in summer season in Dhaka, Bangladesh. The minimum average temperature as well as the maximum temperature for three experiment days were recorded 25.6°C and 30.2°C respectively. Under direct sunlight, there was a greater intensity of the incident light because direct sunlight is a great source of full spectrum exposure. All the formulations displayed gradual decrease in potency upon six hours of undisturbed exposure (**Fig. 2**). The average of all the potency decrease was 28.36% with a standard deviation of 4.87%. Among all the lighting conditions, direct sunlight gave the highest potency reduction in all of the formulations (**Table 1,2,3,4**).

The greatest reduction was provided by the formulation C (33.44%±0.22%). Most of the samples of Formulation C displayed somewhat consistent reduction in their potency (very low standard deviation). This can be attributed to their content uniformity and uniform mixing of the formulation ingredients. On the other hand, rest two formulations displayed different potency reduction in different samples (greater standard deviation). This can be due to the difference in content of the API. Other reasons may include formulation variables, processing variables, moisture content as well as the thickness and quality of the packaging.

Two incandescent lighting conditions i.e. 25 watt bulb and 40 watt bulb produced gradual decrease in potency of all the formulation samples under six hours exposure (**Fig. 3 and 4**). Under the 25 watt lighting condition for six hours exposure (**Table 3**), the mean potency reduction was 19.72%±4.83% for

all the formulations. The individual potency reductions were 15.64%±1.90%, 17.40%±0.97% and 26.12%±0.71% for formulation A, B and C respectively. Exposure of formulation A, B and C under 40 watt lighting condition for six hours (**Table 4**), the mean potency decrease was 25.40%±4.17% and the individual potency decreases were 25.18%±2.34%, 21.52%±3.92% and 29.49%±0.15% respectively. For both the lighting conditions, like two lighting conditions discussed above, Formulation C displayed greatest amount of potency declination (**Table 3 and 4; Fig. 3 and 4**). As it was expected, exposure under 25 watt bulb produced lesser potency reduction and 40 watt bulb exposure provided greater potency change. Since the photolytic degradation of a pharmaceutical product depends directly on the intensity of the incident light, greater potency reduction by 40 watt exposure is not unlikely<sup>7</sup>.

In each of the cases, temperature was recorded i.e. 33°C after six hours exposure of 25 watt bulb and 35°C after six hours exposure of 40 watt bulb. It is obvious that there was not much difference in two temperatures. It can be concluded that the photo-degradation in these cases, is influenced by the intensity of the light mostly, since the temperatures were fairly close in both lighting conditions.

**CONCLUSION:** After discussing and analyzing all the outcomes of this study, it can be said that the pharmaceutical companies should focus on the light-protective packaging of diazepam. This study recommends that the existing transparent packaging of diazepam tablets should be replaced and marketed in light protective packaging to ensure optimum potency as well as the desired therapeutic effect.

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**CONFLICT OF INTEREST:** No

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