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Research Article

In-Vitro Release Kinetics of Clonazepam from Five Brands of Clonazepam Available in Bangladesh Using UV Spectroscopic Analysis in Deionized Water Media

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ABSTRACT

Background and Objective: The purpose of this study was to determine the *in vitro* release kinetics of five brands of Clonazepam tablets available in the local pharmaceutical market of Bangladesh. **Methodology:** In this study, five widely prescribed brands C1, C2, C3, C4 and C5 were chosen. All of these brands were 0.5 mg Clonazepam with strip packaging. The dissolution was carried out using USP apparatus-II and the analysis was performed with the UV spectroscopy. To find out the release kinetics, K_0 (for zero order), K_1 (for first order), K_h (for Higuchi model) were determined. The R^2 values for each kinetics were also determined which indicated the linearity of release kinetics for each brand. **Result:** The study found no brand to follow the zero-order and first order kinetics mostly except Higuchi's drug release profile. The brands showing different R^2 values for Higuchi Drug release profiles are C1 (R^2=0.9843), C2 (R^2=0.9548), C3 (R^2=0.9726), C4 (R^2=0.9578), C5 (R^2=0.9334) which were the highest amongst the R^2 values comparing to zero order and first order values. **Conclusion:** It is concluded that the available Clonazepam tablet brands available in Bangladesh generally follow the Higuchi's drug release kinetics.

Keywords: Clonazepam, Dissolution, release kinetics, *In-vitro* drug dissolution, drug release equations.

1.0. Introduction

Benzodiazepines, remarkably a potent antipsychotic drug class, mechanizes by enhancing the effect of neurotransmitter gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor[1] and hence it acts on the central nervous system, produce sedation, hypnosis, muscle relaxation, down regulate the anxiety levels and also effectively used in the treatment of 'Lennox-Gastaut syndrome'[2]. Clonazepam, a major oral tranquilizer of choice classified under the Benzodiazepines was patented in 1964 followed by successful marketing after being invented by 'Roche'[3] which began to be indicated for treating mainly epilepsy, panic attacks, insomnia, anxiety, seizures, muscle disorders etc[4][5]. Due to its widespread availability, Clonazepam has also been used as abusive purposes such as leading to various physical and psychic dependence causing withdrawal reactions, showing major side effects like drowsiness, dizziness, depression, fatigue[6], coordination and movement obstacles etc.[7] Clonazepam is highly contraindicated for patients with severe liver, kidney, lung and narrow angle glaucoma[8]. The pharmacokinetic property shows the dose-dependency throughout the whole dose-regimen and typically the elimination half-life is around 30 to 40 h[9]. The inhibitory action of central nervous system by clonazepam may be potentiated by alcohol, narcotics, barbiturates, nonbarbiturates, antianxiety agents etc. In addition, Clonazepam has a tendency to interact with opioids causing respiratory depression when combined together[10]. Both Cytochrome P-450 and CYP3A have an important role in clonazepam reduction and oxidation[11].

In the pharmaceutical industry, drug dissolution testing is conducted to determine *in vitro* drug release information for both quality control

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purposes and drug development[12]. To determine the rate of dissolution, when a solid oral dosage form like tablets or capsules are taken, the effectiveness depends on the whether the drug dissolves in the fluids of the gastrointestinal tract before it is absorbed into the systemic circulation[13]. Dissolution is how an active product ingredient is extracted out of the dosage form into the solution within the gastrointestinal tract whereas the term *'in vitro'* defines the testing method of how a drug after being released from its bounded form is mixed in a particular dissolution media examined by the use of a USP dissolution apparatus in a dissolution tester[14]. Release kinetics is nothing but a mathematical model that is used to evaluate the kinetics and mechanism of the performance of drug release with time. The best fitted release kinetic model will be the one with the highest correlation coefficient (R^2) value[15].

This study showed the method of determining the release of kinetics of five different renowned brands of Bangladesh. Dissolution was carried out to determine the percent drug release data and with the help of various mathematical release kinetic models, the best fitted model was determined for each of the brands from their highest R^2 value.

2.0. Materials and methods:

This *in vitro* study was conducted at the Advanced Pharmaceutical analysis laboratory of East West University during the period November-December, 2016 with the materials 0.5 mg Clonazepam tablets, deionized water media, dissolution apparatus, UV spectrophotometer and analytical balance. Five brands which were undergone dissolution study in deionized water media were collected from the various local pharmacy shop of Bangladesh. All the five brands were randomly coded as C1, C2, C3, C4 and C5 respectively and used aluminium strip packaging with 0.5 mg Clonazepam. The in vitro dissolution study was performed with the help of a dissolution apparatus. The tablets were undergone a dissolution study for 60 minutes period and the samples taken at an interval of 10 minutes were analyzed under UV spectrophotometer to build release kinetic models graphically in Microsoft Excel Software 2016.

In-vitro dissolution study:

The *in vitro* dissolution study was conducted by USP type II apparatus at 75 rpm with a temperature of 37±0.5°C that was divided into six section assembly. Dissolution was carried out in 900 mL deionized/distilled water in each of the assembly. Ten milliliters of dissolution medium was withdrawn by pipette during 1 h duration of dissolution study. It was analyzed at 273 nm after filtration. The percent drug release data was determined separately with the help of UV spectroscopic analysis by keeping time along x-axis against the variables of drug releases in accordance with the equation along the y-axis concerned such as zero order, first order and Higuchi equation models to calculate the R^2 value from where the highest one concludes about the release kinetics model[16][17].

Determination of Release kinetics: Generally, for an immediate solid dosage form like Clonazepam, zero order, first and Higuchi equation for drug release is applied to determine the actual release kinetics.

Equation for zero order kinetics: The equation for zero order drug release is- $\mathbf{Q_t = Q_0 + K_0 t}$ where, Q_0 = initial amount of drug, Q_t = cumulative amount of drug release at time't', K_0 = zero order release constant, t= time in hours. It describes the system where the rate of drug release is independent of its concentration of the dissolved substance. A linear graph may be obtained keeping the cumulative percent of drug release at y-axis and time (in hours) along x-axis[18][19].

Equation for first order kinetics: The equation for first order drug release is- $\mathbf{Log\ Q_t = Log\ Q_0 + K_t / 2.303}$ **or** $\mathbf{Q_t = Q_0.e^{-kt.}}$ Here, Q_0 = initial amount of drug, Q_t= cumulative amount of drug release at time't', K= first order release constant, t= time in hours. In this case, the drug release rate depends on its concentration. A

graph is plotted between the time taken on X axis and log of cumulative percentage of the remaining drug to be released on Y axis that gives a straight line. [18] [19]

Equation for Higuchi drug release: The equation for Higuchi drug release is- $\mathbf{Q = K_H t^{1/2}}$. Here, Q= cumulative amount of drug release at time't', K_H= Higuchi constant, t=time in hours. It describes the drug release as a diffusion process. A graph is plotted between the square root of time taken on the x-axis and the cumulative percentage of drug release on y-axis and it gives a straight line[18][19].

The method of this study was based on constant cumulative percent release of drug with time. In an excel spreadsheet, the necessary data were input and scattered diagram of both the standard curve as well dissolution curves were graphically produced to obtain the equations which resemble the standard equation of a slope, **y=mx+c** and also to obtain the values of R^2. Calculating the values of x in the equation ultimately led us to the percent drug release data that helped to build the release kinetic model based on zero order, first order and Higuchi release equations graphically represented by line scattered diagram.

3.0. Results and Discussion:

A standard curve is usually prepare using standard Active product ingredient (API) of the dosage form but in this case due to the non-availability of raw API, the 0.5 mg tablet of a renowned brand C4 was dissolved in the dissolution media (deionized water) in a 50 ml of volumetric flask preparing a solution of 40µg/ml. After proper filtration, the effect of excipients was nullified out as much as possible and hence with the data obtained from the UV spectroscopy analysis, a standard curve was prepared using a concentration range of 0-40 microgram per milliliter that provided an equation **y = 0.0146x + 0.0025** with a value of **(R2= 0.9996)** clearly indicating the proficient linearity of the curve. (Fig. 1)

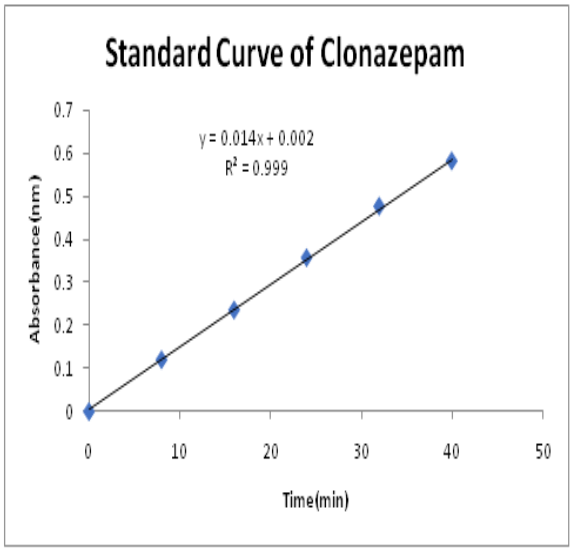
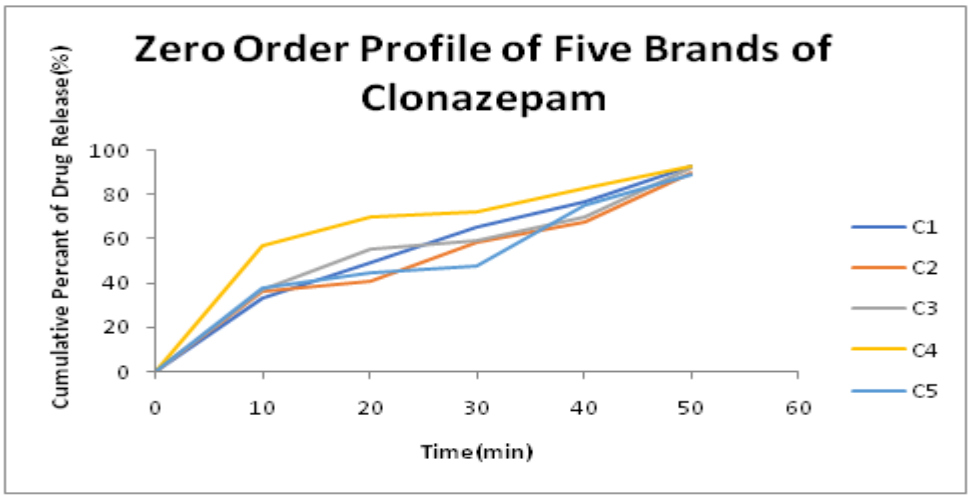


Figure 01: Standard curve of Clonazepam prepared using brand C4.

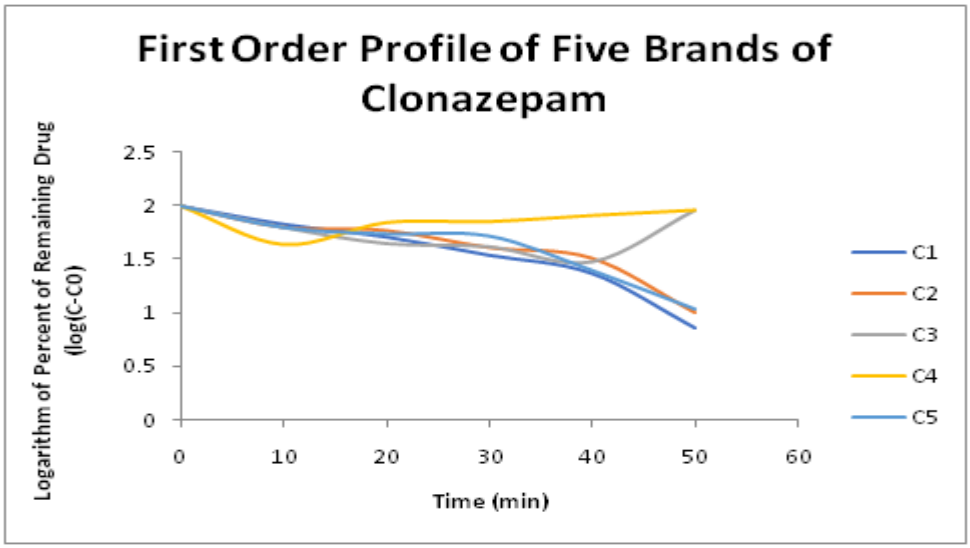
Table 01: Cumulative percentage of drug release from different brands of Clonazepam

Time (minute)	C1	C2	C3	C4	C5
0	0	0	0	0	0
10	32.72	36	36.51	56.57	37.71
20	49.09	40.5	54.95	69.42	44.57
30	65.45	58.5	58.64	72	48
40	76.36	67.5	69.71	82.28	75.42
50	92.72	90	91.84	92.57	89.14



a)

Figure 2a: Zero order plots of five brands of Clonazepam tablets.



b)

Figure 2b: First order plot of five brands of Clonazepam tablets.

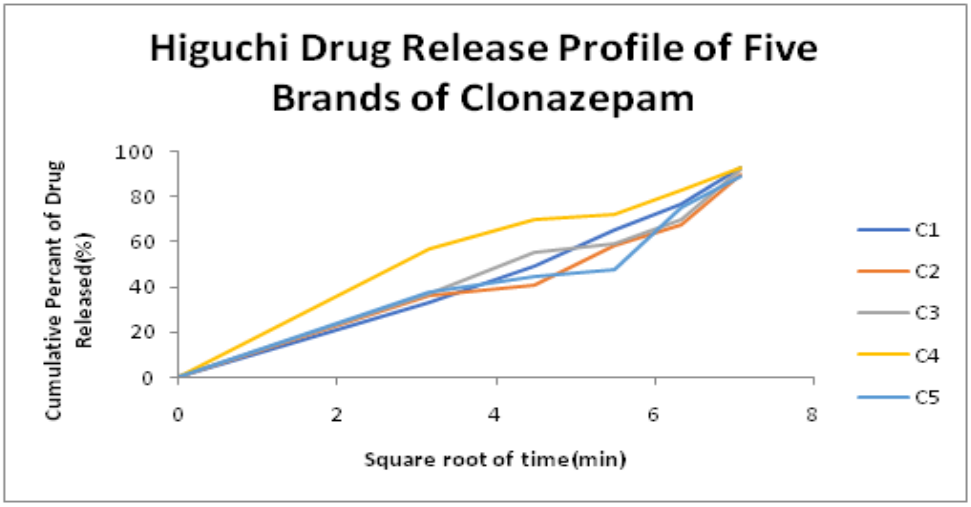


Figure 3: Higuchi plot of five brands of Clonazepam tablets.

Clonazepam is a commonly used anti-depressant drug which is commercially available in pharmaceutical market manufactured by various pharmaceutical companies of Bangladesh. This study was aimed at conducting an experiment based on *in-vitro* drug dissolution to evaluate the release kinetics of drugs from the drug matrix. Three mathematical equations were used to come to a conclusion about interpreting the release kinetic model of the drugs from their solid matrix. It is noteworthy that the R^2 value obtained from the curves of zero order, first order and Higuchi drug release profile provided an idea about the drug release kinetics.

The cumulative drug release vs. time has been shown in Table 1. A zero order drug release is always constant over a particular time period[20]. Hence, a zero order graph was plotted with the help of cumulative drug release pattern against time. After 10 minutes, all the brands were released at a range of around 32 to 36 %. After 50 minutes had passed of drug dissolution, all the brands (C1, C2, C3, C4 and C5) provided a cumulative drug release of 92.72%, 90%, 91.84%, 92.57% and 89.14% respectively. The zero order release rate constants (K_0) were calculated from the zero order equation as well as the correlation coefficient values (R^2 values) that were determined from the graph. From the Fig. 2a, it was evident that brand C4 displayed least R^2 value of 0.7825 whereas the R^2 value 0.9669 of brand C1 was found maximum (Table 2). On the other hand, the release rate constants ranged from 0.84 to 1.2.

According to the first order drug release kinetic model, the rate of drug release is directly proportional to the drug concentration which remains in the drug matrix i.e. it's a concentration dependent process[21][22]. In this regard, the graph of first order release kinetic model was prepared by plotting the time (min) on x-axis and percent (%) logarithm of the remaining drug on y-axis. The release rate constants were found in Fig. 2b and it was within the range of values (-0.0205 to 1.04). The R^2 values were also obtained from the graph where brand C4 having R^2 value of 0.0707 was the least and that of brand C1 (R^2=0.9212) was the highest indicating sufficiency of the linearity of first order release kinetics (Table 2).

A Higuchi's model assumes that diffusion of dissolved drug through the matrix is the rate-limiting stage while determining the release kinetic model[23]. In the Higuchi plot, square root of time was plotted along x-axis against the cumulative percent of drug plotted along y-axis. The release rate constants for this model were in the range of 10.34 to 13.11. In Fig 3, the highest R^2 value was provided by the brand C1 (R^2=0.9843) and the least linearity was displayed by the brand C5 (R^2=0.9334) (Table 2).

Table 02: Comparison of R^2 values of five different brands of Clonazepam to determine the release kinetic model of drug release.

Brands	R^2 values zero order	R^2 values first order	R^2 values Higuchi's
C1	0.9669	0.9212	0.9843
C2	0.9505	0.8719	0.9548
C3	0.9228	0.0968	0.9726
C4	0.7825	0.0707	0.9578
C5	0.9296	0.8836	0.9334

4.0.

5.0. Conclusion:

The R^2 value is the indicative of the release kinetics for the different brands. The highest R^2 values for a particular brand that was obtained either from Zero order/First order/Higuchi's drug release profile were assumed to have been

released following that particular release kinetic equation. For C1, the highest R^2 value=0.9843 is for Higuchi's equation so it will be released from its solid matrix in the dissolution media following Higuchi's mathematical model presumably. Similarly, the highest R^2 values for C2, C3, C4 and C5 are 0.9548, 0.9726, 0.9578 and 0.9334 that clearly indicated that all of the rest brands of Clonazepam also followed Higuchi's equation profile of release kinetics respectively.

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