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Comparative *in vitro* Dissolution Study of Clonazepam Tablets of Bangladesh by UV-Visible Spectrophotometry

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ABSTRACT

This study was aimed to assess the pharmaceutical equivalence of four brands of clonazepam tablets available in Bangladesh using *in vitro* dissolution study by UV spectrophotometry. Dissolution study in water was carried out using USP type-2 paddle apparatus. Other quality control tests like weight variation, disintegration time and assay were also performed according to the established methods. Almost all the samples attained 85% drug dissolution within 15 minutes. The assay revealed that all brands contained around 94-107% (w/w) of labeled chemical content. All brands complied with the official specifications for disintegration time. Dissolution results of all the test products were compared to that of the reference product with difference factor (*f1*) and similarity factor (*f2*). Apart from brand B, the dissolution profiles of other brands showed no significant variations. The study indicates that brands C, D and E were equivalent to the reference brand A so they may be prescribed interchangeably.

Keywords: Clonazepam, dissolution, disintegration, pharmaceutical equivalence

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INTRODUCTION

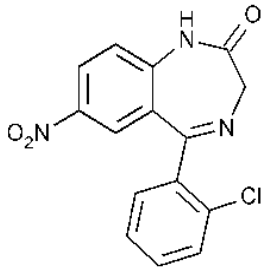
Clonazepam is an extremely potent benzodiazepine that is predominantly used to treat panic disorders, different types of seizures like myotonic or atonic seizures, absence seizures, mania[1,2]. It has also been reported to possess broad spectrum of activity against different types of epilepsy like photosensitive epilepsy and also appears to be highly effective in patients having proved resistance against other antiepileptic drugs[3]. This agent is also indicated to use for the treatment of parasomnia in children[4]. Pharmacodynamically, clonazepam exhibits manifestation of anticonvulsant, anxiolytic, sedative as well as central muscle relaxation action[5,6]. It is available in oral, parenteral dosage forms and pediatric drops[7].

The process of extracting drug from its solid-state matrix dosage form into solution within the gastrointestinal tract is referred as dissolution in pharmacokinetics. This process performs a significant role in releasing drug substance from the drug product in the body for its subsequent absorption[8]. Dissolution largely depends on the physicochemical properties of drugs as well as the formulation and process of manufacturing of the drug product[9]. Hence, it is essential to undertake constant dissolution analysis of marketed drug products to assure the quality of medicines[10].

Multiple pharmaceutical companies manufacture Clonazepam under different brand names in Bangladesh. When the generic products are bioequivalent with the innovator brand, only then generics are altered with the branded version[11]. *In vivo* bioequivalence research is conducted on animals and human whereas *in vitro* research is performed in dissolution equipment, in simulated biological conditions. Bioavailability depends on drug dissolution and its permeability across GIT, pharmaceutical equivalence of these drugs is determined by *in vitro* dissolution[12].

According to Biopharmaceutics Classification System (BCS), clonazepam belongs to Class II where drug permeability is high but solubility of drug is low[7]. This is used as the basis for the setting of *in vitro* dissolution specifications. The comparative dissolution study has been accomplished for many drugs in different countries. However, no studies were found to report a comparative dissolution profile on selected brands of clonazepam. Therefore, the present study was executed to evaluate the quality attributes of five brands of "clonazepam 2 mg tablets" in Bangladesh by *in vitro* investigation. To analyze clonazepam, different methods are available which might contain limitations to some extent. So, an economic, rapid UV Visible scheme was used for analysis of dissolution. Product information of Clonazepam is given at Table 1.

Table 1. Product Information

Product Name	Clonazepam 2 mg tablets
Molecular Weight	315.71 g/mol
Molecular Formula	$C_{15}H_{10}ClN_3O_3$
Chemical Name	5-(2-chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one
Chemical Structure	

METHODOLOGY

Drugs and Chemicals

Five brands of clonazepam 2mg tablets were purchased from a local drug store of Dhaka, Bangladesh. To maintain the confidentiality among the brands and restrict further use of the data of comparative study for commercial purposes, the brands' and manufacturers' names were kept undisclosed and thus the brands were randomly coded with alphabets. The drugs were checked for the manufacturer license no., DAR No., batch number, manufacturing and expiry dates (Table 2).

Table 2. List of Brands of Clonazepam 2mg tablets used *in vitro* analysis

Brand	Manufacturer	Batch No.	Mfg. Date	Exp. Date
A	'A' Manufacturing Company, Bangladesh	10003	June, 20	May, 22
B	'B' Manufacturing Company, Bangladesh	20004	March, 20	February, 22
C	'C' Manufacturing Company, Bangladesh	20D0224	May, 20	April, 23
D	'D' Manufacturing Company, Bangladesh	9004	April, 20	March, 22
E	'E' Manufacturing Company, Bangladesh	0E02409	August, 20	July, 22

Standard API of Clonazepam was collected from Sigma-Aldrich. Methanol (RCI LABSCAN Limited, Thailand) and Acetonitrile (SAMCHUN, Korea) obtained were of HPLC grades.

Instruments and Devices

BK-RC3 Dissolution Tester (Biobase, China), BK-BJ2 Disintegration Tester (Biobase, China), BA2004N Analytical Balance (Biobase, China), BK-UV 1800 Spectrophotometer (Biobase, China), PH-10S pH Meter (Biobase, China), UC-20A Water Bath Sonicator (Biobase, China) were used for the experiments.

Calibration of Dissolution Apparatus and UV- Spectrophotometer

UV/VIS spectrophotometer was internally calibrated and the calibration process was carried out to evaluate for absorbance and wavelength controls, stary light limit, photometric linearity, resolution power and appropriateness of baseline and sample cells. The calibration of the dissolution apparatus was performed half-yearly on a routine basis following the standard procedure recommended by United States Pharmacopoeia (USP) as per the General Chapter <711>[13]. As Reference Standard, USP Prednisone Tablets RS and USP Prednisone RS were used for the performance verification test. The dissolution test was performed according to Table 3. After manual sampling, the release of prednisone was determined through UV/VIS spectrophotometer.

Table 3. Specifications for calibration.

Parameters	Specifications
Medium	Degassed purified water
Volume	500 mL
Rotation Speed	50 rpm
Bath Temperature	37 ± 0.5 °C
Time Point	30 minutes
Absorbance Wavelength	242 nm

Method Validation

In this study the dissolution method was validated for Clonazepam 2 mg Tablets with official medium (water) by UV spectrophotometer at a wavelength of 254 nm using 10 mm cell. The validation was done according to ICH Q2 (R1) guideline[14]. Method validation characteristics were system suitability, specificity, linearity, accuracy, precision, intermediate precision and filter compatibility. Summary of the method validation is given at Table 4.

Table 4. Method validation report summary

Validation parameters	Acceptance criteria		Results
System suitability & Specificity	RSD (%) of five replicate absorbance of standard should be ≤ 2.0		≤ 2.0
	Interference of diluent and placebo		No interference
Linearity	R^2 value should be ≥ 0.999	50%, 80%, 100% 120% and 150% (0.011 mg/mL as 100%)	0.999
Accuracy	Average Recovery (%) of three sample at each recovery level within 98.0 to 102.0	50% (0.0055 mg/mL)	99.2
		100% (0.011 mg/mL)	99.9
		150% (0.0165 mg/mL)	100.2
Precision	RSD (%) six tablets after 45 min should be ≤ 2.0		1.1
Intermediate precision	RSD (%) six tablets after 45 min in different day with different analyst should be ≤ 2.0		1.0
	RSD (%) 12 tablets of both Precision Intermediate precision should be ≤ 2.0		1.1
Filter Compatibility	There should be no interference of filter paper		Filter paper does not adsorb the active.

Determination of Weight Variation

Twenty tablets of each brand were weighed individually with the analytical balance. The average weights and relative standard deviations were calculated and compared[15].

Disintegration test

Six tablets from each brand were taken and placed in disintegration chamber containing distilled water at 37°C within the tester. The time required for total disintegration and passing of the tablet entirely through the sieve was recorded[16].

Dissolution test

All dissolution tests in this study were performed using USP Apparatus 2 with official dissolution medium (water) as recommended in USP monograph for Clonazepam tablets. Three tablets of each brand were simultaneously placed in

900 mL of demineralized water at 37±0.5°C and allowed a rotation at 75 rpm. 10 ml of solution was withdrawn at 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes with replacement of equal volume of media. After each withdrawal, samples were filtered through #41 Whatman filter paper and assayed by UV visible spectrophotometer with a 10 mm quartz cell. A standard curve of pure API was derived at 254 nm, which was determined first through spectrum mode[17,18].

Assay

Ten clonazepam tablets were weighed and crushed; equivalent weight of tablet was weighed out and dissolved in methanol. This solution was then further diluted to get the desired concentration of 0.2mg/ml (working standard). The absorbance was determined through UV Visible spectrophotometer at 254 nm (90-110%)[18].

Drug release kinetics

In order to evaluate the drug release kinetics from the tablets, results of the dissolution study were fitted with different kinetic equations including:

Zero-order kinetics:

First-order kinetics:

Higuchi model:

Korsmeyer–Peppas kinetics: $Q_t/Q_o = Ktn$

Hixson-crowell model:

where, K_o, K_1, K_H, K_{HC} and K_{kp} indicates zero-order, first-order, Higuchi, Hixson-crowell and Korsmeyer–Peppas rate constants respectively, Q_t/Q_o means fraction of drug released at time t, K means rate constant and n means release exponent. The kinetics that gives the highest regression coefficient (R^2) value is considered as the best fit model[19].

Data analysis: Data were expressed as Mean ± standard deviation. The dissolution profiles were analyzed with difference factor (*f1)* and similarity factor (*f2*).

RESULTS AND DISCUSSION

Table 5 represents results obtained from the evaluation of tablets including weight variation, disintegration time and assay. The homogeneity of the tablet weight of each unit is evaluated to measure weight uniformity. An average weight of twenty tablets of the different brands was found in the range of 82-162 mg. All the clonazepam tablets were within the acceptable range (± 10%) of

the average weight (for tablets that weigh below 130mg), which is set by United States Pharmacopeia (USP)[7].

Table 5. Summary of quality attributes of Clonazepam 2mg tablets

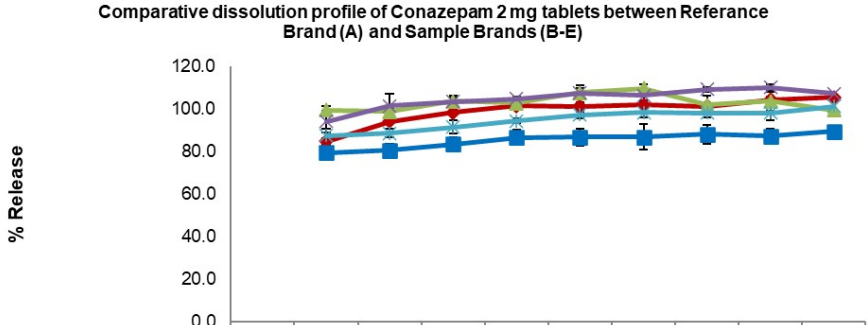
Brand	Weight variation (mg)		Disintegration Time (sec.) Mean ± SE (n=6)	Assay %
	RSD	Mean ± SE (n=20)		
A	1.2	135.08 ± 0.36	79 ± 4.32	107.3
B	1.8	81.53 ± 0.33	23 ± 1.45	98.7
C	1.0	102.95 ± 0.22	30 ± 2.17	98.0
D	0.7	162.65 ± 0.26	37 ± 3.54	106.8
E	2.3	83.07 ± 0.43	20 ± 0.80	94.1

Disintegration times of all tablet formulations were within the acceptable limit. USP compounding compendium states that uncoated and plain-coated tablets should disintegrate within 30 minutes. The use of different disintegrating agents in different formulation or products may result in different disintegration times[20]. Moreover, the production process of the tablets has discrete influence on disintegration of dosage form such as granulation techniques, compression force used for preparing tablets.

Dissolution Profiles

According to USP, the oral solid dosage form of clonazepam (tablet) should exhibit 85% dissolution in 60 minutes[7]. The result of the dissolution test of marketed clonazepam tablets were within the specification of pharmacopoeia. Almost all the brands released more than 85% of API within 15 minutes, except for brand B.

Dissolution process is influenced by various factors. For tablet formulation, nature of excipients used and the disintegration rate are very crucial points. Generally, four primary factors are involved in the intestinal absorption of drug substance from a solid oral dosage form. These include intestinal transit, membrane permeability, available surface area and the concentration profile of drug in the lumen. The solubility attributes of a drug can be predicted from the operation of dissolution test in a selected medium. Therefore, the drug readily disintegrates and exerts its therapeutic effect.



	Time (min)	5	10	15	20	25	30	35	40	45
Reference Brand (A)		84.7	94.0	98.3	101.3	101.0	101.7	101.0	104.3	105.3
Brand (B)		79.4	80.4	83.4	86.4	86.7	86.7	88.0	87.0	89.4
Brand (C)		99.3	98.7	103.6	102.7	107.6	109.6	101.7	103.6	99.3
Brand (D)		94.0	101.3	103.3	104.6	107.3	106.3	109.0	110.0	107.0
Brand (E)		87.2	88.4	91.4	94.3	97.0	98.3	98.0	98.0	101.0

Figure 1: Comparative dissolution profile of Clonazepam 2 mg tablets between Reference Brand (A) and Sample Brands (B-E). Data were presented as mean ± standard error mean. Dissolution profiles (% dissolution) of brand B-E (n=3) were analyzed in five minutes intervals up to 45 minutes and compared with the reference brand A.

Dissolution profiles for marketed clonazepam tablets exhibit behavior according to the recommendation of USP (Figure 1). The fact that clonazepam is a BCS class II compound can explain the observation. The rate-limiting step for absorption of clonazepam is dissolution. The presence and the amount of disintegrating agents at varied extent such as sodium starch glycolate or povidone in different formulations may affect the dissolution attributes of tablets manufactured by different manufacturer. In addition, usage of different coating materials such as polyethylene glycol, ethylene cellulose etc. can also have significant effect on disintegration as well as dissolution or drug release in aqueous medium. The hydrophobic nature of clonazepam limits its dissolution in water.

Table 6. Results of Different models in terms of r^2, slope and intercept.

Model	Parameters	A	B	C	D	E
Zero order model	R^2	0.51491	0.46293	0.46922	0.47364	0.29488
	Slope	2.4643	2.005	2.4957	2.4914	1.8971
	Intercept	46.036	41.782	51.35	50.743	54.97
First order model	R^2	0.84179	0.61266	0.34487	0.58214	0.29915
	Slope	-0.0653	-0.0226	-0.0415	-0.054	-0.0333
	Intercept	1.5791	1.6547	0.9017	1.2065	1.2799
Higuchi model	R^2	0.79136	0.74178	0.74307	0.7542	0.54528
	Slope	17.557	14.586	18.049	18.068	14.826
	Intercept	22.25	21.388	26.333	25.597	32.129
Korsmeyer-Peppas model	R^2	0.86429	0.81524	0.81252	0.82982	0.60949
	Slope	66.824	55.69	68.739	69.024	57.087
	Intercept	15.688	15.76	19.545	18.586	25.925
Hixson-Crowell model	R^2	0.91225	0.55219	0.80154	0.71639	0.01256
	Slope	0.1956	0.0562	0.2035	0.1968	0.0218
	Intercept	0.7298	0.9764	1.6455	1.9144	2.753

From the comparison shown in Table 6, it was found that all the brands were well fitted to the Hixson-Crowell model, except brand E. This model assumes that the release rate is limited by the dissolution rate of drug particles and not by diffusion[21]. Hixson-Crowell equation is used to interpret the dissolution data of dispersible or immediate release dosage formulations. Therefore, a higher correlation coefficient indicates that change in surface area and diameter of particles during the process of dissolution have an effect on drug release[22].

Table 7. Calculated difference factor *(f1)* and similarity factor *(f2)* for all Clonazepam 2mg tablets

Samples	*f1*	*f2*
Brand (B)	14	36
Brand (C)	5	54
Brand (D)	6	54
Brand (E)	4	58

For clonazepam, difference factor *(f1)* and similarity factor *(f2)* were used to evaluate the dissolution profiles and pharmaceutical equivalence (Table 7). The difference factor *(f1)* is proportional to the percentage (%) difference be-

tween the two profiles at each time point and is a measurement of the relative error between the two curves, whereas the similarity factor *(f2)* is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity between the two curves. The following equations were used to perform the calculation for clonazepam comparison. Here, *Rt* represents the percentage value of drug dissolved at time t obtained with the reference drug, n depicts the number of collection times considered for the calculation of *f2* and *Tt* is the dissolved percentage value of test drug (similar) at time *t*[23].

$$f_1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|]/[\Sigma_{t=1}^{n} R_t]\} \cdot 100$$
$$f_2 = 50 \cdot \log\{[1+(1/n)\Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \cdot 100\}$$

All the *f1* values are within the range of 1 to 15. Apart from brand B, *f2* values of other brands were above 50 thereby demonstrating an acceptable dissolution profile according to the established criterion. The rate-limiting step of drug absorption from the gastrointestinal tract is dissolution from the tablet; other factors are related to the manufacturing process. The evaluated brands were formulated in a way that enhances the solubility of the drug, releasing 80% of API in 5 minutes. Therefore, a large portion of clonazepam is absorbed in the stomach, remaining is absorbed in the small intestine. The absorption may vary in other parts of the gastrointestinal tract. Since the value of *f2* is greater than 50 for brands C, D and E, it can be concluded that these products show similar dissolution to that of reference brand A[23].

The results obtained in this study indicate that except brand B, all the other brands of Clonazepam (2mg) tablets complied with the USP specifications and can be considered to be equivalent to the reference product. It can be assumed that these tablet formulations may have similar bioavailability, however further *in vivo* study is required to support this presumption.

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STATEMENT OF ETHICS

The paper is exempt from ethical committee approval.

CONFLICT OF INTEREST STATEMENT

All authors agreed on the article before submission and had no conflict of interests.

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AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors Mohammad Mustakim Billah and Razwanur Rahman Tushar designed, coordinated and supervised the project and also performed the statistical analysis. Sadman Sakib Bin Rashed performed in vitro experiments and participated in acquisition of data. Nusrat Jahan Vabna drafted the manuscript and Fairuza Ahmed analyzed the data, performed the drug release kinetics and critically revised the manuscript. Laila Jahan provided technical support for the experiments. All authors read and approved the final manuscript.

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