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Original Article

Preparation and In-Vitro Evaluation of Novel Orally Disintegrating Tablet of Clonazepam Containing Extracted Starch as Natural Disintegrant

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A R T I C L E I N F O

A B S T R A C T

In this present investigation , oral disintegrating tablets(ODT) of clonazepam were prepared by direct compression method with a view to enhance patient compliance. In addition to it, starch obtained from different natural sources like oats,barley,millets,wheat etc are used as a natural disintegrant to minimize the adverse affects. Five formulations were designed and The prepared batches of tablets were evaluated for hardness, friability, drug content uniformity, wetting time, dispersion time and in vitro drug release. FTIR study was performed to interpret the drug ,excipient compatibility . Based on these in-vitro evaluation results, the formulation F-3 prepared by using starch obtained from oats was considered the optimal one. as it showed the maximum drug release of 99.1% in 30 minutes and showed the least wetting time 21 second and disintegration time 24second

Key Words: Wetting test, Clonazepam, Direct Compression Method.

1. INTRODUCTION

Oral drug delivery is currently the gold standard in the pharmaceutical industry where it is regarded as the safest, most convenient and most economical method of drug delivery having the highest patient compliance[3]. Formulation of a convenient dosage form for oral administration, by considering dysphagia especially in case of geriatric and paediatric patient leads to poor patient compliance. To

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troubleshoot such problems a new dosage form known as orally disintegrating tablet (ODT), has been developed which rapidly disintegrate & dissolve in saliva and then easily swallowed without need of water which is a major benefit over conventional dosage form.

It shows increased bioavailabilty due to pregastric absorption and improved safety due to low risk of choking or suffocation during oral administration.

Clonazepam belongs to benzodiazepine class. Clonazepam is rapidly and completely absorbed after oral administration. Clonazepam having absolute bioavailability about 90%. shows maximum plasma concentrations within 1 to 4 hours after oral administration. Clonazepam is highly metabolized with less than 2% unchanged Clonazepam being excreted in the urine. The elimination half-life of Clonazepam is typically 30-40 hours

Clonazepam is a benzodiazepine indicated for seizure disorder and epilepsy[1,2]. Patients suffering from seizures will have problem in swallowing the tablet In such cases, oral disintegrating tablets will be an effective solution for patient compliance and efficient medicine regimen.

The main aim of the project work is to formulate and evaluate the oral disintegration tablets by using disintegrants extracted from natural sources such as potato, barley, millets, oats and wheat. Here we tried to reduce the disintegration time by taking the optimum concentration of starch extracted from various natural sources
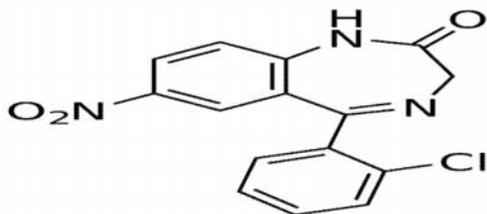

Fig 1: Structure of Clonazepam

2. MATERIALS AND METHODS

Clonazepam was procured as a gift sample from Dr. Reddy's lab, Hyderabad, India. PVPK30 , lactose, mannitol were purchased from M.S.N lab, Hyderabad, India. Magnesium stearate and talc were purchased from Granules India , Hyderabad.

Experimentals Methods

Preparation of standard stock solution :

Standard stock solution of Clonazepam was prepared by dissolving accurately weighed quantity of clonazepam(10 mg) in 100ml of methanol with sonication and transferred it to 100ml of volumetric flask. Volume was made up to the mark with methanol for obtaining standard stock solution of 100µg/ml.

Preparation of calibration curve of clonazepam:

Aliquots of 0.4-2.8 ml of stock solutions were transferred to series of 10 ml volumetric flasks, and volume made up to mark with methanol to give concentration range (4-28 µg/ml). The absorbance was measured at absorption maximum of 309 nm against methanol as blank and calibration curve was plotted.

Drug-excipient compatibility studies:

Incompatibilty between drug and excipient can alter stability and bioavailability of drugs, thereby, affecting its safety and/or efficacy. Study of drug excipient compatibility is a important process in the development of a stable dosage form. A number of techniques can be used to indicate the drug/excipient interactions. Therefore, the pure drug and the formulations mixed with polymers were subjected to infra-red (IR) studies. The study was performed by preparing KBR pellets with the help of KBR press, taking 1mg sample in 100mg KBR. The scanning range was 400-4000 cm^{-1} and there solution was 1cm^{-1}

Method of isolation of starch:[5,6]

In this present investigation starch was isolated from five different powder sources (millets, oats, barley, potato, wheat) of natural origin. 250gm of each powder was taken in five separate beaker. sufficient amount of water was added to form a thick suspension. the suspension was filtered using strainer into a 1000ml beaker. ,Than the beaker was kept aside for 1 hour by adding sufficient amount of water to it. gradually the starch settled down at the bottom of beaker. the supernatant liquid was separated and again the beaker was filled with water. the same procedure was repeated for 4-5 times ,then the purified starch was collected, than the extracted starch was kept in hot air oven at a temperature below 60 degree Celsius. Obtained starch powder was confirmed by iodine test and used for the formulation of ODT tablets.

Pre-formulation studies:

Preformulation studies of powder blend

Flow properties of powder blend play a pivotal role in developing and designing a optimized formulation. In order to determine the flow characteristics of powder blend different test such as angle of repose, bulk density, tapped density, compressibility index and Hausner's ratios were determined according to the standard procedures given in USP.

Design and Development of clonazepam oral disintegrating tablet[4]

Clonazepam oral disintegrating tablet was formulated by direct compression method. As a key ingredient the disintegrants extracted from natural sources such as barley, millet, oats, potato and wheat in similar concentration was incorporated in the formulation of ODT tablets, PVP K-30 used as binder, lactose was used as diluent, Mannitol was used as taste masking agent . All ingredients used were passed through a # 100 sieve, weighed and blended. All the formulations details were represented in Table-1.The individual formulations were compressed by using 9 mm flat

faced punches using in a rotary tablet press (Rimek minipress, model RSB-4, M/S: Karnavathi engineering, Ahmadabad).

Table 1: Composition of ODT tablet of Clonazepam

Ingredients(mg)	F1	F2	F3	F4	F5
Clonazepam	2mg	2mg	2mg	2mg	2mg
Lactose	35mg	35mg	35mg	35mg	35mg
Potato Starch	40mg	-	-	-	-
Millets Starch	-	40mg	-	-	-
Oats Starch	-	-	40mg	-	-
Barley Starch	-	-	-	40mg	-
Wheat Starch	-	-	-	-	40mg
PVPK30	40mg	40mg	40mg	40mg	40mg
Mannitol	29mg	29mg	29mg	29mg	29mg
Magnesium stearate	2mg	2mg	2mg	2mg	2mg
Talc	2mg	2mg	2mg	2mg	2mg
Total	150mg	150mg	150mg	150mg	150mg

Physical evaluation of oral disintegrating tablet of Clonazepam[11]

Weight variation test

The weight variation test was done by weighing 20 randomly selected tablets individually, calculating the average weight and comparing the individual tablet weights to the average.

$$W\% = \frac{Wav - W1}{Wav} \times 100$$

Hardness test:

The hardness of the tablets were determined using Monsanto Hardness tester. It is expressed in kg/cm2. Six tablets were randomly picked from each formulation and the mean and standard deviation values were calculated.

Friabiltiy

The friability test was conducted to find out the powder loss in the tablets using an Roche friaballator. Twenty tablets were selected from each batch and any loose dust was removed with the help of a soft brush. The tablets were initially weighed and transferred into friabilator. The drum was rotated at 25 rpm for 4 minutes after which the tablets were removed. Any loose dust was removed from the tablets as before and the tablets were weighed again . The percentage friability was then calculated by the formula

$$F\% = \frac{Win - Wf}{Win} \times 100$$

Drug content uniformity:

Five tablets of each formulation were weighed and powdered. The quantity of powder was equivalent to 2 mg.

The equivalent weight of clonazepam was transferred into 100 ml volumetric flask and by using the extracting solvent and samples were analyzed spectrophotometrically by using UV/ Visible spectrophotometer at 309nm.

Wetting test:

Wetting time of dosage form is related to with the contact angle. Wetting time of the ODT is another important parameter, which needs to be assessed to give an insight into the disintegration properties of the tablet. Lower wetting time implies a quicker disintegration of the tablet. The wetting time of the tablets can be measured by using the simple procedure. Five circular tissue papers of 10cm diameter are placed in a petridish. Ten milliliters of water soluble dye solution is added to petridish. A tablet is carefully placed on the surface of the tissue paper. The time required for water to reach upper surface of the tablet is noted as the wetting time.

Disintegration test: The time for disintegration of ODTs is generally <1min and actual the disintegration time that patients can experience ranges from 5 to 30s. The standard procedure of performing disintegration test for these dosage forms has several limitations and they do not suffice the measurement of very short disintegration times. The disintegration test for ODT should mimic disintegration in mouth with in salivary contents.

In-vitro drug release study:[13]

Dissolution studies were carried out for all the formulations combinations by employing USP - II paddle method and 900ml of pH 6.4 phosphate buffer as the dissolution medium. The medium was allowed to equilibrate to temp of $37°C \pm 0.5°c$. Tablet was placed in the vessel and the vessel was covered the apparatus was operated for 30minutes in pH 6.4 phosphate buffer at 50 rpm. At definite time intervals of 5 ml of the aliquot of sample was withdrawn periodically and the volume replaced with equivalent amount of the fresh dissolution medium. The samples were analyzed spectrophotometrically at 309nm using UVspectrophotometer. And this dissolution data was further treated for kinetic modeling.

Drug release kinetics study :

There are several linear and non-linear kinetic models to describe release mechanisms and to compare test and reference dissolution profiles are as follows:

Zero order kinetics Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation:

$W0 - Wt = K0t$ Where W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the amount of drug in the pharmaceutical dosage form at time t and k is proportionality constant. Dividing this equation by W_0 and simplifying: $ft = k0t$

Where ft = 1 –(Wt/ W0) and **ft** represents the fraction of drug dissolved in time t and k0the apparent dissolution rate constant or zero order release constant .

First order kinetics: This type of model to analyze drug dissolution study was first proposed by Gibaldi and Feldman and later by Wagner. The relation expressing this model: Log Qt= Log Q_0+ K_1t/2.303

Where Qt is the amount of drug released in time t, Q_0 is initial amount of drug in the solution and K_1 is the first order release rate constant .

Korsmeyer Peppas model : Korsmeyer developed a simple semi empirical model, relating exponentially the drug release to the elapsed time (t). $Qt/Q = K_k t^n$ Where K_k is a constant incorporating structural and geometric characteristic of the drug dosage form and n is the release exponent, indicative of the drug release mechanism

Higuchi Model:
$Qt = K_H t^{1/2}$ Where Qt = the amount of drug released at time t and K_H = the Higuchi release rate;
This is the most widely used model to describe drug release from pharmaceutical matrices. A linear relationship between the square root of time versus the concentration indicates that the drug release follows strict Fickian diffusion .

3. RESULTS AND DISCUSSION

Compatibility study:
Spectra of the pure drug, excipient and physical mixture of drug were recorded in between 400-4000 wave number (cm-1). The FTIR spectral analysis showed that there is no appearance or disappearance of any characteristic peaks of pure drug clonazepam and in the physical mixture which confirms the absence of chemical interaction between drug and polymers.

Pre formulation study results:
In this investigation. All five formulation were subjected for various pre-compressional evaluations such as angle of repose, bulk and tapped density, compressibility index and Hausner's ratio. The finding of pre-compression evaluation was given in **Table-2,** Angle of repose was found to be ranging from 21.29^0 to 30.68^0 all formulation shows good flow property . Carr's index was found to be ranging from 10.52% to19.29 % for the powders of all the formulations.

Post compression results:
The results of physical evaluation of tablets is given below in **Table-3** The tablets of different batches were found uniform with respect to hardness within the range of 3.59 to 4.01 kg/cm2. Another measure of a tablet's strength is friability. Results of friability test were also has been found within limit. In weight variation test, the pharmacopoeial limit for percentage deviation for tablets of more than 150 mg is ±5% and all the formulations were found to comply

with the specifications given in I.P. for weight variation test. Good uniformity in drug content was found among the formulations, and percentage of drug content was more than 95%. All the tablet formulations showed acceptable pharmaco-technical properties.

Disintegration and Wetting study:
The tablets of different batches were subjected to disintegration and wetting test. the results are presented in Table-4, **F3** formulation containing 40mg starch as disintegrant ,extracted from oats showed the least wetting time 21 second and disintegration time 24second. **F5** formulation showed maximum wetting time 25 second and disintegration time 31 second

In - vitro drug release study:
The release profile of clonazepam from different batches of formulated ODT tablets were illustrated in Table-5 and drug release profile graph is given in Figure-6 . among all the formulation F3 showed the maximum drug release of 99.1% in 30 minutes.F2 formulation showed low drug release of 88% in 30minutes .

Drug Release Kinetics study:
The kinetic data of all the formulations are graphically represented in figure7 to 10 . In order to determine the mechanism of drug release form the formulations , the in-vitro dissolution data was fitted to Zero order, First order, Higuchi plot and Korsemeyer peppa's plot was drawn for optimized formula and interpretation of release exponent value (n) was calculated. The results of R2 for zero and first order was 0.740 and 0.940 respectively. Based on that we have confirmed that the optimized formulation followed first order release. Higuchi's model was applied to the in-*vitro* release data, linearity was obtained with high 'r' value indicating that drug release from the oral disintegrating tablets through diffusion. The *in-vitro* release data was further fitted to Krosmeyer-Peppas model which is generally used to analyze the release mechanism when more than one type of release phenomenon is operational.

Table 2: Pre-compression Results of Clonazepam ODT tablets

Fomulations	Angle of Repose(Degree)	Tapped Density(g/mL)	Bulk Density(g/mL)	Hausner'sRatio (%)	Carr's Index(%)
F1	26.02	0.67	0.58	1.08	18.77
F2	21.29	0.63	0.55	1.15	11.33
F3	30.12	0.52	0.42	1.23	19.22
F4	26.22	0.57	0.46	1.23	19.29
F5	30.68	0.63	0.57	1.10	10.52

Table 3:Physical evaluation of Clonazepam ODT tablets

FORMULATION	Weight variation	Diameter(mm)	Hardness(kg/cm²)	Friability(%)	Uniformity content
F1	150	8.00	3.59	0.46	99.11
F2	151	8.00	4.01	0.59	99.97
F3	150	7.98	3.58	0.86	99.12
F4	153	8.01	3.99	0.53	96.34
F5	151	7.09	3.89	0.78	95.32

Table 4:Disintegartion time , In-vitroWetting time, Dispersion time

FORMULATION	Disintegration Time(Sec)	In-vitroWetting Time(Sec)	In-vitro Dispersion

			Time(Sec)
F1	28	24	31
F2	25	22	29
F3	24	21	28
F4	26	22	28
F5	31	25	32

Table 5:Drug release profile of formulation F1 to F5

TIME(Minutes)	F1	F2	F3	F4	F5
0	0	0	0	0	0
5	69	45	53	23	44.3
10	78	51	79	45	65.76
15	80	63	87.4	61	78.31
20	85	75	89	75	85.01
25	87	82	95.03	82	92.4
30	89	88	99.1	87	95
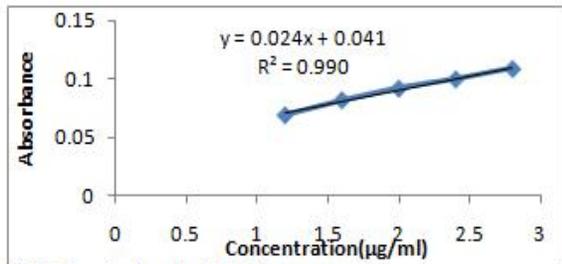
Fig 2: Standard graph plot of Clonazepam.
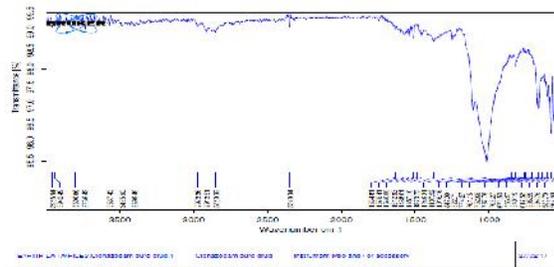
Fig 3: FTIR of Clonazepam pure drug
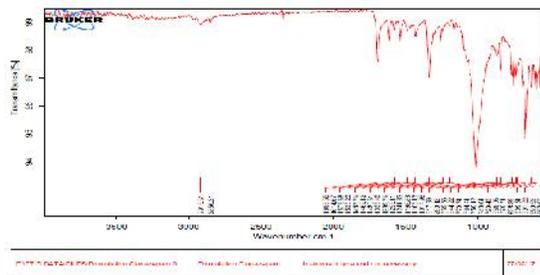
Fig 4: FTIR of Optimized F-3 Clonazepam formulation
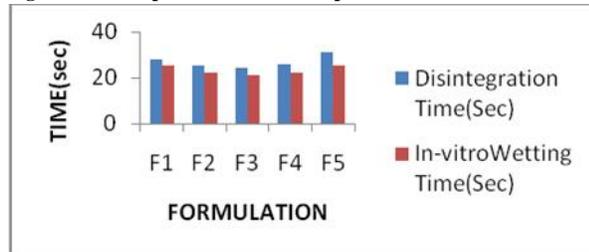
Fig 5: Disintegration time and Wetting time of ODT formulations F1 to F5
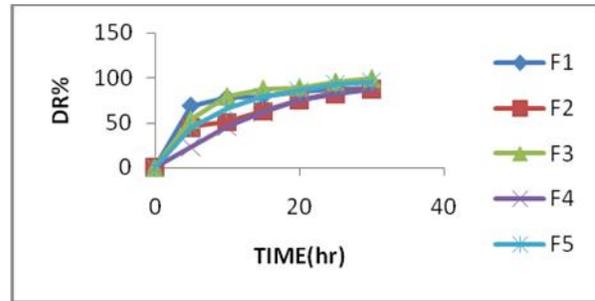
**Fig 6: In-vitro drug release of ODT formulations(F1to F5)**
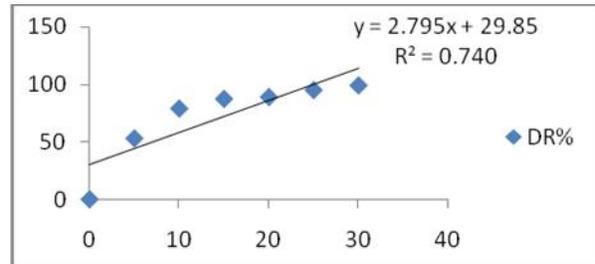
**Fig 7: Zero order drug release plot for Optimized F3 formulation of Clonazepam**
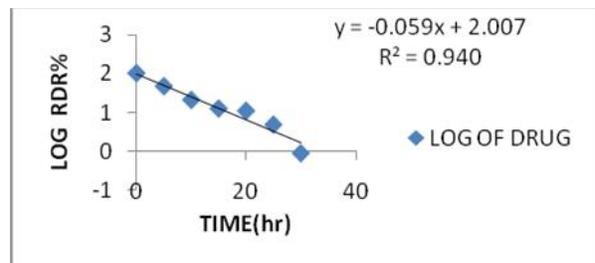
Fig 8 :First order drug release plot for Optimized F3 formulation of Clonazepam
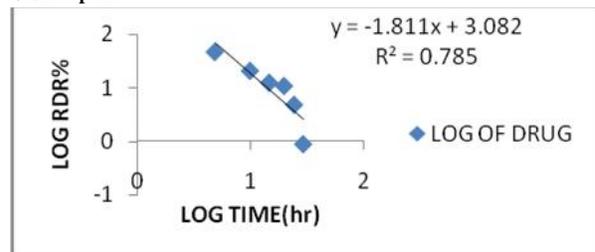

Fig 9 :Korsemeyer peppa's drug release plot for Optimized F3 formulation of Clonazepam
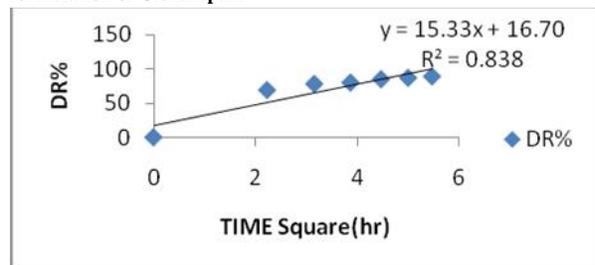

Fig 10 :Higuchi drug release plot for Optimized F3 formulation of Clonazepam

Fig 11 : Process of Extraction of starch from different natural sources

Fig 12 : Extracted starch from natural sources

Fig 13 : Formulated ODT tablets of Clonazepam
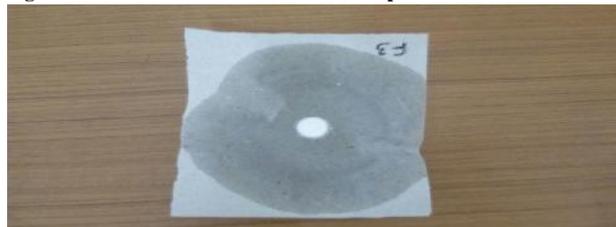
Fig 14 : Wetting process

Fig15: Dispersion process

4. CONCLUSION

The study was undertaken with the aim to design and evaluate the clonazepam oral disintegrating tablets using starch as disintegrant extracted from various natural sources. From the above results and discussion, it is concluded that F3 formulation of the oral disintegrating tablet containing natural disintegrants extracted from oats is best and ideal formulation (F3) that fulfils all the requirement of oral disintegrating tablet.

From the kinetic studies it was known that all the formulations released at first-order rate and were even found to be optimum for stability studies.

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