WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 5, 1251-1267. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF CLONAZEPAM ORALLY DISINTEGRATING TABLETS, 1 MG

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Article Received on
08 Jan. 2018,

Revised on 29 Jan. 2018,
Accepted on 19 Feb. 2018,

DOI: 10.20959/wjpr20185-11324

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ABSTRACT

Clonazepam is a benzodiazepine indicated for seizure disorder, panic disorder and epilepsy. Patients suffering from seizures will have difficulty in swallowing the tablets or will be reluctant to take the tablets or will spit the administered tablet. In such cases, orally disintegrating tablets will be an effective solution for patient compliance and efficient medicine regimen. In the present research, orally disintegrating tablet of Clonazepam was made by aqueous wet granulation process. Pearlitol Flash and Microcrystalline Cellulose were used as diluent. Crospovidone was used as disintegrant. Strawberry Flavor and Aspartame were used as flavoring and sweetening agents. Sodium Lauryl Sulphate was used as a wetting agent. Colloidal Silicon Dioxide was used as glidant. Talc and Magnesium Stearate were used as lubricants. The prepared tablets were evaluated for weight, thickness, hardness, friability, disintegration time and dissolution. Prepared tablets showed disintegration time of less than 30 seconds and drug dissolution of about 75% achieved within 30 minutes. The prepared tablets were stability tested at 40°C / 75% RH for 3 months and were found to be stable. Prepared orally disintegrating tablets of Clonazepam 1 mg was found to be bioequivalent under fasting and fed conditions with the internationally marketed product.

KEYWORDS: Wet granulation and bioequivalence under Fasting and Fed.

INTRODUCTION

Clonazepam belongs to benzodiazepine class. IUPAC name of Clonazepam is 5-(2-chlorophenyl)-1, 3-dihydro-7-nitro-2H-1, 4-benzodiazepin-2-one. Clonazepam is a light yellow crystalline powder with a molecular weight of 315.72. Clonazepam is rapidly and completely absorbed after oral administration. The absolute bioavailability of Clonazepam is about 90%. Maximum plasma concentrations of Clonazepam are reached within 1 to 4 hours after oral administration. Clonazepam is approximately 85% bound to plasma proteins. 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 pharmacokinetics is dose-dependent throughout the dosing range.[1-10]

RATIONALE

Patients suffering from seizure, epilepsy and panic disorder will not be cooperative because of the underlying disorder and associated physical and mental disturbance. Clonazepam is an insoluble drug, for achieving rapid dissolution and onset of action, Sodium Lauryl Sulphate was chosen as a wetting agent. Clonazepam belongs to class of drugs having narrow therapeutic window and also the dose being less, direct blending and dry granulation process were avoided. To achieve better content uniformity and intimate mixing with pharmaceutical excipients, aqueous wet granulation process was selected. Strawberry flavor was selected as a flavoring agent because of wide acceptance from pediatrics to geriatrics cadre. Aspartame was selected as sweetener since it is 200 times sweeter than sucrose; one of the most widely used artificial sweetener and phenyl alanine content per tablet was considered before finalizing the level of aspartame usage in formulation. Ionic disintegrants like Sodium Starch Glycolate and Croscarmellose Sodium were not used in formulation because of contribution of sodium content per tablet. Nonionic disintegrant, Crospovidone was selected as disintegrant. Pearlitol Flash – a novel co-processed excipient of Mannitol and Maize starch was selected along with Microcrystalline Cellulose as diluents. Colloidal Silicon Dioxide was selected as glidant to improve the flow property of blend. Talc and Magnesium Stearate were selected as Lubricants.

LITERATURE REVIEW

Shirsand *et al.* (2008) prepared the orally disintegrating tablets of Clonazepam, 2 mg by direct blending and compression process. Three (3) compositions were finalized with different disintegrant and the composition includes, Clonazepam – 2 mg, Crospovidone – 15

mg / Croscarmellose Sodium – 15 mg / Sodium Starch Glycolate – 15 mg, Microcrystalline Cellulose – 52.5 mg, Aspartame – 3 mg, Sodium Stearyl Fumarate – 1.5 mg, Talc – 3 mg and Mannitol – 73 mg with a total tablet weight of 150 mg. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

Sarasija Suresh *et al.* (2009) prepared orally disintegrating tablets of Clonazepam, 2 mg by direct blending and compression process. The composition includes, Clonazepam – 2 mg, Crospovidone – 3 mg, Microcrystalline Cellulose – 60 mg, Aspartame – 3 mg, Sodium Stearyl Fumarate – 1.5 mg, Pine apple flavour – 1.5 mg, Talc – 3 mg and Mannitol – 76 mg with a total tablet weight of 150 mg. The composition was optimized by factorial design. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

Shirsand *et al.* (2011) prepared orally disintegrating tablets of Clonazepam, 2 mg by direct blending and compression process followed by Sublimation method. The composition includes, Clonazepam – 2 mg, Croscarmellose Sodium – 7.5 mg, Camphor – 60 mg, Aerosil – 3 mg, Aspartame – 3 mg, Sodium Stearyl Fumarate – 1.5 mg, Pineapple Flavour – 1.5 mg, Talc – 3 mg and Mannitol – 68.5 mg with a total tablet weight of 150 mg. Once the tablets were prepared they were subjected to dry heat in hot air oven at 60°C for 6 hours. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

Sarasija Suresh *et al.* (2011) prepared Fast Dissolving Effervescent Tablets of Clonazepam, 2 mg. The composition includes, Clonazepam – 2 mg, Crospovidone – 12 mg, Sodium Bicarbonate – 36 mg, Citric Acid – 36 mg, Aspartame – 3 mg, Flavour (Pineapple) – 1.5 mg, Talc – 1.5 mg, Sodium Stearyl Fumarate – 3 mg, Mannitol – 52 mg with a total tablet weight of 150 mg. The process involved direct blending and compression. But before mixing all the ingredients, Sodium Bicarbonate and Citric Acid were individually dried in hot air oven at 80°C for 2 hours to remove residual or absorbed moisture. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

Thakkar Hardik *et al.* (2011) prepared orodispersible tablets of Clonazepam, 2 mg by direct blending and compression process. The finalized composition includes, Clonazepam – 2 mg, Crospovidone – 6 mg, Microcrystalline Cellulose 102 – 38 mg, Mannitol (Pearlitol SD 200) – 94 mg, Aspartame – 3 mg, Magnesium Stearate – 2 mg, Talc – 5 mg with a total tablet weight

of 150 mg. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

Prajapati Amit *et al.* (2011) prepared solid dispersion by spray drying of Clonazepam along with PVP K-30 and Lactose. The spray dried materials was finally blended with Ammonium bicarbonate, Crospovidone, Microcrystalline Cellulose and Magnesium Stearate and compressed into orally disintegrating Tablets. The tablets were then kept in hot air oven at 40 to 50°C till all the ammonium bicarbonate sublimes. The tablets were characterized by *in-vitro* disintegration and dissolution tests.

Swati C Jagdale *et al.* (2011) prepared solid dispersions of Clonazepam in polyethylene glycol 4000 and 6000 by employing various techniques in the ratio of 1:1, 1:0.5 and 1:0.25 with the aim to increase its aqueous solubility. Drug polymer interactions were investigated using Fourier transform infrared spectroscopy and UV spectroscopy. By these determinations no drug-polymer interactions were evidenced. Solubility and dissolution study were performed and both solubility and dissolution rate of the drugin these formulations were increased. Finally, tablets were produced by direct compression and dissolution tests were realized in order to evaluate the dissolution profiles. The results show that the tablets can be classified as immediate release dosage forms due to clonazepam fast release, and such release was dependent on the amount of superdisintegrant (cross-povidone and Doshion P544) in the formulation.

Swati C Jagdale *et al.* (2012) prepared solid dispersions of Clonazepam using Gelucire 50/13 to increase the water solubility of API. Orally Disintegrating tablets were prepared from the optimized batches (kneading method) of solid dispersion, using Crospovidone and Doshion P544 resin as superdisintegrant. The tablets were characterized by *in-vitro* disintegration and dissolution tests. The study of the MDTs showed disintegration times in the range 32.0±0.85 to 20.0±1.30 sec.

Sai Padmini Bolla *et al.* (2014) prepared Oro dispersible tablet of Clonazepam, 2 mg. The composition includes Clonazepam – 2 mg, Osimum basilicum – 10 mg, Microcrystalline Cellulose – 100 mg, Magnesium Stearate – 2 mg, Talc – 2 mg, Mannitol – 83 mg, Aspartame – 1 mg with a total tablet weight of 200 mg. Osimum basilicum powder was prepared by powdering the basil seeds of Osimum basilicum and the powder is defatted using Petroleum Ether at 60-80°C and activated for 1 hour at 100-120°C. Then the material is soaked in 1/6th

part of Chloroform-Water for 24 hours. The material concoction is then filtered through muslin cloth. To the filtrate an equal volume of alcohol (95%) was added to precipitate the excipient. Then the final mass is dried till constant weight in hot air oven at 50°C. The tablets were made by direct blending and compression process by mixing all the above listed materials. The Physico-chemical attributes of the end product met the required specification of orally disintegrating tablets.

NOVELTY

Clonazepam is a poorly soluble drug. Based on the literature information, Dry granulation / hot melt extrusion granulation / solid dispersion by fusion or solvent evaporation / ion-exchange complexation / direct compression process will not be explored. No non-aqueous solvent will be used. To achieve good organoleptics, effective solubilisation and good content uniformity wet granulation process using Sodium Lauryl Sulphate in Water will be done for manufacturing the Clonazepam Orally Disintegrating Tablets, 1 mg. The formulation will be fully optimized with respect to composition and process to ensure good blend flow, tableting, organoleptics and stability. The finalized drug product will be evaluated for comparable dissolution profile and bioequivalence against the internationally marketed product.

OBJECTIVE

To develop suitable manufacturing process for Clonazepam Orally Disintegrating Tablet 1 mg that exhibits comparable physico-chemical characteristics and stability with that of internationally marketed product.

MATERIALS AND METHODS

Excipients and Reagents

Clonazepam was obtained from Centaur Pharmaceuticals; Microcrystalline Cellulose (Vivapur Type 101) from JRS Pharma; Sodium Lauryl Sulphate (Stepanol WA100) from Stephane USA; Aspartame from Nutrasweet; Pearlitol Flash EXP from Roquette; Crospovidone (Polyplasdone XL 10) from ISP; Non Bovine Grade Magnesium Stearate (Tablube) from Nitika Chemicals; Colloidal Silicon Dioxide (Cab-O-Sil) from Cabot Sanmar; Strawberry Flavour 052311AP0551 from Firmenich; Talc (Luzenac Talc Ultra Micronized Grade) from Luzenac Pharma; Acetone, Isopropyl alcohol, Methanol, Tetrahydrofuran, Acetonitrile, n-heptane, Anhydrous dibasic ammonium phosphate, Ortho Phosphoric acid, Concentrated Hydrochloric Acid 37%, Sodium Hydroxide Pellets, Potassium Dihydrogen Phosphate, Sodium Acetate Trihydrate and Glacial Acetic Acid were obtained from Merck.

Equipments & Instruments

High Shear Mixer Granulator 10 L of Kevin; Fluid Bed Dryer Processor & Granulator GPCG 1.1 of Pam Glatt; 16 Station Single Rotary Tablet Compression Machine of Cadmach Machinery; Hardness Tester Type: TBH 125 of Erweka; Disintegration Test Apparatus Model ED 2L manufactured of Electrolab; Thickness Tester Vernier Caliper Absolute Digimatic of Mitutoyo; Friabilator EF-1W of Electrolab; Electromagnetic Sieve Shaker EM8-08 Plus of Electrolab; Tap Density Testing Apparatus ETD-1020 of Electrolab; Bottle Sealing Machine (Induction Cap Sealer) Sigma Flex of Electronic Devices; Double Cone Blender with interchangeable bowls of 5L, 15L and 25L of SamsTechnomech; Loss On Drying Mositure Analyzer Model MB 45 of Ohaus; Multi Mill equipped with 1.5 mm screen of Sams Technomech; Comminuting Mill equipped with 0.5 mm screen of Cadmach Machinery; 40°C / 75% RH Stability Chamber of Thermolab Scientific Equiments; HPLC of Waters; Vibrosifter of Gansons Engineering; 6.35 mm Round Shaped Standard Concave Punch Tooling of ACG PAM Pharma Technology; Weighing Machine PS6000/C/1 of LCGC RADWAG; pH Meter H12215 of Hanna Instruments; Lab Stirrer RQ-126D of Remi; Dissolution Apparatus TDT-08L of Electrolab.

Disintegration and Dissolution Parameters

Disintegration test is performed in USP disintegration test apparatus in Purified Water @ 37 ± 0.5°C. Specification: NMT 30 seconds. Dissolution is performed in 900 mL Purified Water. The parameters include, USP-II (Paddle), 50 RPM. Time Points: 5, 10, 15, 30, 45 and 60 minutes. Specification: NLT 75 % (Q) of the labeled amount in 60 minutes.

Method For Assay, Blend Uniformity, Content Uniformity / Uniformity of Dosage Units and Dissolution

Clonazepam is official in USP and the same method is utilized for the determination of Assay, Related Substances, Blend Uniformity, Content Uniformity / Uniformity of Dosage Units and Dissolution.

Method For Bulk Density, Tapped Density, Repose Angle and Friability Measurements

USP guidelines followed in the measurement of Bulk Density, Tapped Density, Compressibility Index, Hausner Ratio, Repose Angle and % Friability measurements.

FORMULATION

Marketed Product Characterization

Clonazepam ODT 1 mg manufactured by Par Pharmaceuticals Inc. was characterized with respect to Inactive ingredients details, Weight, Thickness, Diameter, Disintegration Time and Dissolution Profile and Packaging configuration.

API Characterization

Clonazepam was characterized with respect to Description, Bulk Density, Tapped Density, Compressibility Index, Hausner Ratio, Repose Angle and Particle Size Distribution.

Drug-Excipient Compatibility Study

The possibility of drug-excipient interaction was investigated by HPLC analysis. Drug excipient compatibility study was performed with excipients mentioned above (See 'Excipients & Reagents'). Study was conducted by preparing homogenous mixture of excipient with drug filled in glass vials were exposed to 40 ± 2°C / 75 ± 5% RH and 60°C for 4 weeks and 2 weeks respectively. Samples were analysed for Assay, Total Impurity and Water By Kf.

Formulation And Process

The ingredients listed in the Intragranular part, except Clonazepam and Sodium Lauryl Sulphate were sifted through #20 ASTM sieve. Sodium Lauryl Sulphate solution is prepared in Purified Water, the quantity of Purified Water is 10% of the total quantity of intragranular ingredients except Clonazepam and Sodium Lauryl Sulphate. After getting a clear solution of Sodium Lauryl Sulphate, Clonazepam was added slowly to the solution and controlled stirring was done to avoid foaming and frothing and continued for 45 minutes. The sifted materials were charged in High Shear Mixer Granulator and dry mixed at high impeller and chopper speed for 15 minutes. The dry mixed materials were granulated with Clonazepam-Sodium Lauryl Sulphate dispersion in purified water at high impeller and chopper speed. The Drug-SLS container was rinsed with purified water which is 2% of the total quantity of intragranular ingredients and add the rinsings to the contents of High Shear Mixer Granulator.

The wet granules were dried in Fluid Bed Dryer Processor & Granulator at 60°C till LOD is not less than or equal to 3%. The dried granules were sifted through #40 ASTM sieve. The dried granules retained in #40 ASTM sieve were milled through comminuting mill fitted with 0.5 mm screen at high speed knife forward configuration. The milled granules along with #40

ASTM sieve passed granules were blended together in a Double Cone Blender at 15 RPM for 2 minutes. The extra granular materials except lubricants was sifted through #20 ASTM sieve and blended along with granules of intragranular part in a Double Cone Blender at 15 RPM for 20 minutes. The lubricants were sifted through #40 ASTM sieve and blended with the blended materials in a Double Cone Blender at 15 RPM for 5 minutes. The blend thus prepared was compressed into tablets using 6.35 mm round flat faced bevel edged punch tooling. The drying parameters includes, Inlet Air Temperature – 60° ± 10°C; Exhaust Temperature – 40° ± 10°C; Filter Shaking Interval – 20 ± 5 seconds; Shaking Duration – 5 ± 1 second; Shaking Mode – Auto The blend was characterized with respect to LOD (105°C / Automode), Repose Angle, Bulk / Tapped Density, Hausner Ratio and PSD. Prepared Tablets were characterized with respect to Weight, Thickness, Hardness, Friability, Disintegration Time and Dissolution Profile.

Stability Study

The formulation was subjected to accelerated stability study at 40±2°C / 75±5% RH. 60 Tablets were packed in 60 cc HDPE bottle with 33 mm Child Resistant Cap and induction sealed. Description, Disintegration Time, Dissolution, Assay and Related Substances were studied during stability. In case of dissolution, method followed was USP-II (Paddle), 50 RPM, 900 mL, Purified Water @ 37 ± 0.5°C. Time Points: 5, 10, 15, 30, 45 and 60 minutes. Specification: NLT 75 % (Q) of the labeled amount in 60 minutes.

In vivo Study

An open-label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, crossover, bioequivalence study of Clonazepam ODT 1 mg with that of internationally marketed product was conducted in 12 healthy adult human subjects under fasting and fed condition. The study protocol was prepared and approval from Independent Ethical Committee – The Ethical Jury, Chennai was obtained. The studies were conducted in compliance with the ethical principles of the Declaration of Helsinki, the International Conference on Harmonization's Good Clinical Practices guidelines and the guidelines of Indian Council of Medical Research for Biomedical Research on Human Subjects and Good Clinical Practices for Clinical Research in India. Study subjects were screened and enrolled for the study. Enrolled subjects were housed in the clinical facility for 11 hours prior to drug administration until 24 hours post dose. In case of study under fasting condition, after overnight fasting of at least 10 hours, a single oral dose of either tests or reference product

was administered i.e. the assigned formulation was placed on the subjects tongue and instructed to hold the tablet to disintegrate for 30 seconds and later to swallow it with about 240 mL of water in sitting posture. Then the subject will be fasted for at-least 4 hours post dosing. In case of study under fed condition, after overnight fasting of at least 10 hours, subjects were served standard high-fat, high-calorie breakfast 30 minutes prior to administration of investigational product. After providing a high-fat, high-calorie breakfast, a single oral dose of either test or reference product was administered to the subjects i.e. the assigned formulation was placed on the subjects tongue and instructed to hold the tablet to disintegrate for 30 seconds and later to swallow it with about 240 mL of water in sitting posture. The pre-dose 0 hour blood sample was collected before dosing and post dose samples were collected at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 12.00 and 24.00 hours. Subjects were provided standard diet and continuously monitored for well-being and safety throughout the study. The concentration of Clonazepam in plasma samples were analysed using validated analytical method. Pharmacokinetic and statistical analyses were performed on obtained drug concentration data. Healthy, willing human volunteers between 18 and 45 years were selected on the basis of medical history, physical examination (including but may not be limited to an evaluation of the cardiovascular, gastrointestinal, respiratory and central nervous systems) vital sign assessments, 12-lead electrocardiogram (ECG), X-ray, and clinical laboratory assessments, urine screen for drugs of abuse and alcohol breath test. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. Pharmacokinetic analysis was performed using WinNonlin® software version: 5.3 of Pharsight Corporation, USA for the following pharmacokinetic parameters Cmax, AUC0-t, AUC0-∞, Tmax, Kel, t½ Kel_Lower, Kel_Upper and AUC_% Extrap_obs. Analysis of variance (ANOVA) consistent with two one-sided test for bioequivalence, ratio analysis and 90% confidence intervals for ratio of least square mean of Ln-transformed data of Cmax, AUC0-t and AUC0-∞ for test and reference products were calculated by using SAS® statistical software version 9.1.3 from SAS Institute Inc, USA. For Clonazepam, the 90% confidence interval of the relative mean Cmax, AUC0-t and AUC0-∞ of the test and reference product should be between 80.00% and 125.00% for log-transformed data.

RESULTS AND DISCUSSION

Marketed Product Characterization

The following were the marketed product characterization details based on which the Quality Target Product Profile was decided.

Table 1: Marketed Product Characterization.

Particulars	**1 mg**
Description	White, round, flat-faced, beveled edge tablets, debossed "K8" on one side.
Excipients	Mannitol, Aspartame, Crospovidone, Silicon dioxide, Sodium lauryl sulphate, Sorbitol, Talc and Magnesium Stearate
Diameter	6.35
Avg. Weight (mg)	99.83
Avg.Thickness (mm)	2.99
Avg. Hardness (kP)	2.98
% LOD (105°C / Automode)	2.94
Disintegration Time (secs)	21.42
Time (min)	**Dissolution (mean % drug dissolved)**
5	48.8
10	64.3
15	75.4
30	88.0
45	91.2
60	92.7
Pack Details	HDPE bottle of 60 Tablets and Peel off Alu-Alu blister of 6 tablets.

API Characterization: From the table below it is evident that the API exhibits poor flow and needs to be improved through formulation.

Table 2: API Characterization.

Particulars	**Details**
Description	Light yellow powder having faint odor with bitter taste
Bulk Density	0.23 g / ml
Tapped Density	0.30 g / ml
Compressibility Index	23.3%
Hausner Ratio	1.30
Repose Angle	38°
LOD (105°C-Auto mode)	0.33%
Particle Size Distribution	d10 0.662 microns
By Malvern Mastersizer®	d50 4.322 microns
	d90 8.008 microns

Drug-Excipient Compatibility Study

Drug Excipient compatibility data shown in Table 3. Suggests that both the temperature and moisture doesn't affect the stability of mixture indicating compatibility of drug with excipients studied.

Table 3: Drug-Excipient Compatibility Study.

API + Excipients	Tests	Initial	Duration / Storage Conditions	
			2nd Week 60°C	4th Week 40°C/75%RH
Clonazepam	%ASSAY	99.09	98.16	100.82
	%TI	0.006	0.017	0.009
	%Moisture	0.06	0.34	0.12
Clonazepam+ Mannitol(1:1)	%ASSAY	92.75	89.87	92.07
	%TI	0.005	0.013	0.007
	%Moisture	0.12	0.08	0.07
Clonazepam + Pregelatinized Starch (1:1)	%ASSAY	99.58	102.17	98.61
	%TI	0.022	0.018	0.007
	%Moisture	6.05	6.24	5.71
Clonazepam + Crospovidone (1:1)	%ASSAY	97.93	99.10	99.71
	%TI	0.005	0.016	0.009
	%Moisture	6.55	6.37	5.44
Clonazepam+ Aspartame (1:1)	%ASSAY	100.35	97.46	95.78
	%TI	0.019	0.044	0.005
	%Moisture	5.53	4.77	4.99
Clonazepam+ Pearlitol Flash EXP (1:1)	%ASSAY	108.03	103.16	103.99
	%TI	0.006	0.007	0.017
	%Moisture	1.13	1.39	1.00
Clonazepam+ Sodium Lauryl Sulphate (1:1)	%ASSAY	100.66	100.49	101.71
	%TI	0.013	0.025	0.013
	%Moisture	0.12	0.10	0.13
Clonazepam+ Strawberry Flavor 052311AP0551 (1:1)	%ASSAY	100.50	99.96	101.11
	%TI	0.006	0.024	0.261
	%Moisture	1.56	1.03	1.66
Clonazepam+ Microcrystalline Cellulose (1:1)	%ASSAY	99.60	97.64	96.86
	%TI	0.007	0.020	0.016
	%Moisture	4.87	4.15	4.69
Clonazepam+ Colloidal Silicon Dioxide (1:1)	%ASSAY	107.38	106.70	105.65
	%TI	0.007	0.034	0.020
	%Moisture	0.56	0.46	0.61
Clonazepam API+ Non Bovine grade of Magnesium Stearate (1:1)	%ASSAY	103.40	101.92	104.17
	%TI	0.006	0.024	0.014
	%Moisture	2.27	2.19	2.20
Clonazepam + Ultra Micronized Talc (1:1)	%ASSAY	102.92	102.39	105.32
	%TI	0.007	0.028	0.015
	%Moisture	0.22	0.18	0.09
Clonazepam+	%ASSAY	98.38	98.41	101.04

Saccharin Sodium (1:1)	**%TI**	0.013	0.028	0.060
	%Moisture	0.57	0.44	0.44
Clonazepam API+ Acesulfame Potassium (1:1)	**%ASSAY**	98.56	98.40	100.99
	%TI	0.016	0.294	0.131
	%Moisture	3.00	2.87	2.77

Note: TI – Total Impurity; % Moisture determined by Water by Kf.

Formulation Process And Characterization

The composition details and the process details with characterization of blend and tablets were provided in Table 4. From the details it is evident that the composition and process is robust enough to produce the drug product with good physico-chemical and organoleptic attributes. Dissolution profile of the prepared ODT of Clonazepam was compared against the internationally marketed product. The prepared test product complied to the dissolution specification of not less than 75% (Q) in 60 minutes. See Table 5 and Figures 1 for further details.

Table 4: Formulation Process, Blend and Tablet Characterization.

S.No	**Ingredients**	**1 mg**
		mg / tablet
Intragranular Ingredients		
1	Clonazepam	1.000
2	Sodium Lauryl Sulphate	0.015
3	Pearlitol Flash EXP	48.985
4	Microcrystalline Cellulose	4.000
5	Aspartame	1.000
6	Purified Water	QS
Total Intragranular Ingredients		55.000
Extragranular Ingredients		
7	Pearlitol Flash EXP	33.900
8	Crospovidone	8.000
9	Strawberry flavor 052311AP0551	0.100
10	Colloidal Silicon Dioxide	0.500
11	Talc	1.000
12	Magnesium Stearate	1.500
Total Extragranular Ingredients		45.000
Total Tablet Weight		100.000
Blend Characterization Study		
Bulk Density (g/ml)		0.53
Tapped Density (g/ml)		0.77
Compressibility Index (%)		31.2
Hausner Ratio		1.45
LOD of Final Blend (%)		2.06
Repose Angle (°)		27.7
Particle Size Distribution		**% Retained in each sieve**

Sieve # (ASTM)	
20	0.00
40	0.00
60	12.19
80	29.95
100	14.63
140	12.19
200	2.44
Pan	36.60
LOD of Final Tablets (%)	2.80
Turret Speed (RPM)	25
Average Weight (mg)	100.6
Average Thickness (mm)	2.63
Average Hardness (kP)	3.08
Friability (%)	0.16
Disintegration Time (secs)	12-15
Organoleptic Attribute	Pleasant
Capping	× (Not observed)
Lamination	× (Not observed)
Picking	× (Not observed)
Sticking	× (Not observed)

Table 5: Dissolution Profile Comparison of Test Products Vs Marketed Product.

Strength	**Hardness Variation (kP)**	**Mean % Dissolved (n=6 units)**					
		Time (Minutes)					
		5	**10**	**15**	**30**	**45**	**60**
1mg	Reference	48.8	64.3	75.4	88.0	91.2	92.7
	Test	73.7	82.4	91.0	98.5	101.3	102.9
Medium**:** 900 ml, Purified water @ 37 ± 0.5ºC, USP-II (Paddle), 50 RPM, Specification: NLT 75% (Q) in 60 minutes							

Stability Study

The prepared Clonazepam ODT 1 mg were packed in 60 tablets per 60 cc HDPE bottle and capped with 33 mm child resistant polypropylene closure and sealed with induction seal liner. The packed induction sealed bottles were subjected to accelerated stability study at 40°C / 75% RH for 3 months and the prepared drug product was found to be stable. See Table 6 for further details.

In vivo Study

In vivo study was carried out for test formulation and the internationally marketed product. The plasma levels of Clonazepam were determined. The mean concentration-time profiles for the marketed and test products of Clonazepam under fasted and fed condition were shown in

Table 7 and Table 8 and Figure 2 and Figure 3 respectively. T/R ratio shows that the formulated test product was bioequivalent to the internationally marketed formulation.

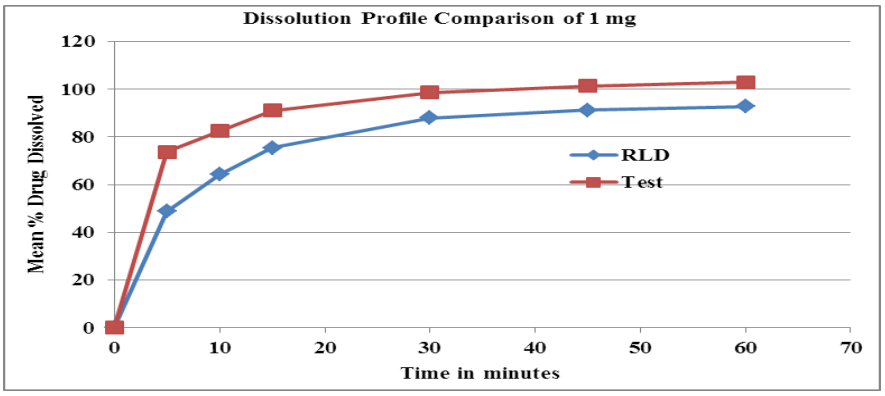


Figure 1: Dissolution Profile Comparison.

Table 6: Stability Results at 40°C ± 2°C / 75 ± 5% RH.

Test	Specification	Initial	1st Month	2nd Month	3rd Month
1 mg					
Description	**	Comply	Comply	Comply	Comply
Disintegration Time (secs)	NMT 30 secs	14	11	11	12
Loss On Drying (%)	NMT 4.0	1.5	2.3	2.1	2.4
Dissolution	NLT 75%(Q) in 60 minutes	98±2.52	98±2.65	97±1.00	95±3.00
Assay (%)	90-110	100.8	98.4	99.9	100.1
Related Substances (%)					
Highest Unknown Impurity	NMT 0.2	0.007	0.034	0.005	0.006
Total Impurities	NMT 2.0	0.007	0.049	0.028	0.040

**White colored round shaped flat faced bevel edged tablets; On stability no morphological / color change observed.

Table 7: Statistical Analysis of Pharmacokinetic Data – Fasted Study.

Product / Statistics	C_{max} (ng / mL)	AUC_{0-t} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	T_{max} (h)
Test Product				
Arithmetic Mean	7.4941	283.2834	331.8177	2.2500
Geometric LS Mean	7.3164	278.0708	324.8921	
Standard Deviation	1.7052	56.3926	69.5425	
Reference Product				
Arithmetic Mean	8.1416	287.0173	343.1658	1.7500
Geometric LS Mean	7.8721	276.5742	333.1791	
Standard Deviation	2.1110	63.8834	75.5883	
T/R (%)	92.94	100.54	97.51	
90% Confidence Interval (T/R)				
Lower Limit (%)	85.62	94.07	94.57	
Upper Limit (%)	100.89	107.46	100.54	
ISCV (%)	18.81%	15.20%	6.96%	

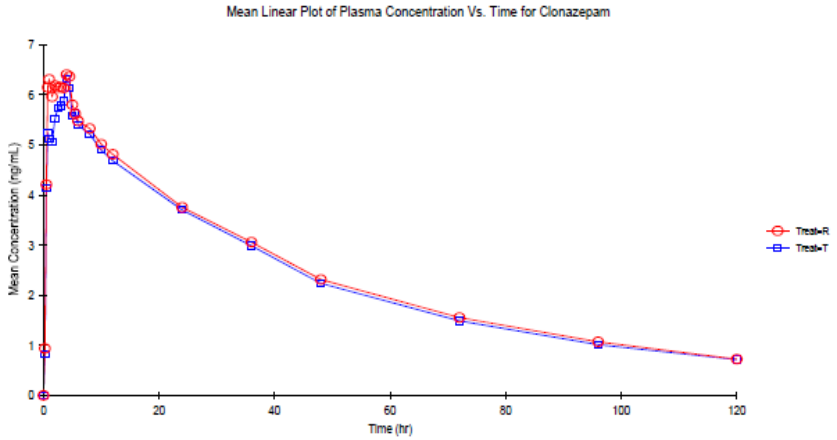
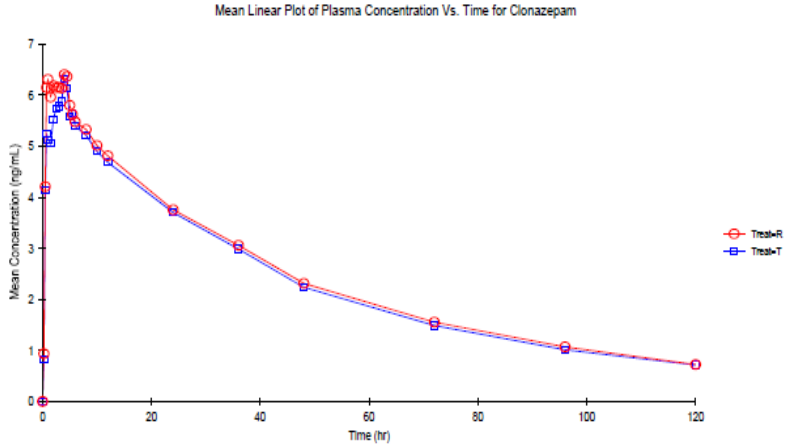


Figure 2: Mean Concentration Time Profile – Fasting Study.

Table 8: Statistical Analysis of Pharmacokinetic Data – Fed Study.

Product / Statistics	C_{max} (ng / mL)	AUC_{0-t} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	T_{max} (h)
Test Product				
Arithmetic Mean	5.5619	263.9917	301.3725	4.5000
Geometric LS Mean	5.4709	260.1201	295.5296	
Standard Deviation	1.1671	47.6148	62.5816	
Reference Product				
Arithmetic Mean	5.8102	261.6408	293.5981	4.5000
Geometric LS Mean	5.7541	258.1364	289.6970	
Standard Deviation	0.8981	43.4670	49.3203	
T/R (%)	95.08	100.77	102.01	
90% Confidence Interval (T/R)				
Lower Limit (%)	91.00	97.68	98.04	
Upper Limit (%)	99.34	103.95	106.14	
ISCV (%)	9.99	7.07	9.03	



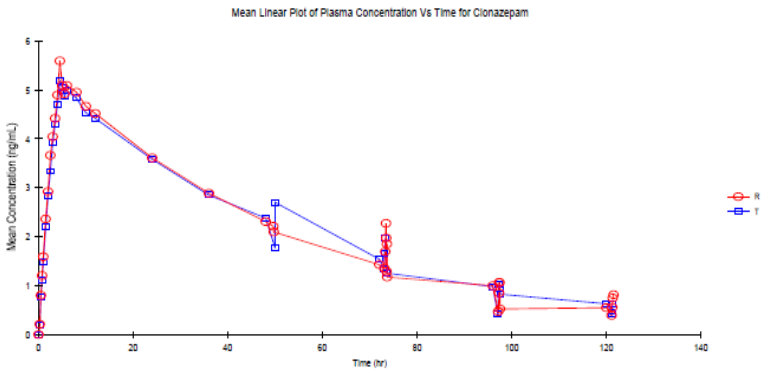


Figure 3: Mean Concentration Time Profile – Fed Study.

CONCLUSION

Orally disintegrating of Clonazepam was successfully formulated by a simple aqueous wet granulation process. Clonazepam is an insoluble drug. Uniformity of dosage units, rapid disintegration and dissolution were fixed as prerequisite design attributes. Sodium Lauryl Sulphate was used as a wetting agent. A combination of Pearlitol Flash EXP and Microcrystalline Cellulose were chosen as diluents. Crospovidone was used as a disintegrant. Strawberry flavor and aspartame were used to impart organoleptic characteristics to the dosage form. Colloidal Silicon Dioxide was used as a glidant. A combination of Ultra micronized talc and Magnesium Stearate were used as lubricants. The finalized formulation showed favorable organoleptic and rapid dissolving property within 30 seconds. No tableting issue was encountered during the compression of batch blend because of ideal lubrication. The blend was characterized by determining bulk density, tapped density, hausner ratio, compressibility index, loss on drying, repose angle and particle size distribution. The finalized composition was subjected to accelerated stability study in HDPE bottle pack and the physico-chemical properties were found to be stable. The prepared ODT of Clonazepam 1 mg was subjected to in vivo bioequivalence study under fast and fed condition against the internationally marketed product and was found to be bioequivalent.

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