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FORMULATION AND EVALUATION OF DISULFIRAM TABLETS, 500 MG

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ABSTRACT

There are few manufacturers available in the market worldwide for Disulfiram Tablets. There is report of high bio variability reported between the marketed, effervescent/dispersible tablets vis a vis immediate release tablets.[1-6] In the present research, Disulfiram Tablets were manufactured by wet granulation process. Manufactured tablets showed good morphology and mechanical strength, without tableting issues. In this research influence of milling and micronization of API on the in vivo bio variability of the drug product was studied and reported.

KEYWORDS: Milling, Micronization, Wet granulation, Core tablet and Bioequivalence under Fasting.

INTRODUCTION

Disulfiram is used for the treatment of chronic alcoholism, cocaine dependence, Giardia infection, Scabies, metronidazole resistant Trichomonas *vaginalis*, proteasome inhibitor, liver cancer, treatment for HIV cure through activating the reservoir of HIV-infected resting CD4 cells, multidrug resistance (Because of P-gp efflux pump modulation and binds to drug substrate binding sites of multiple ABC transporters), fungal infection (Caused by Candida parapsilosis, C. krusei, C. albicans, C. tropicalis, C. glabrata, Aspergillus fumigatus, A. flavus and A.niger), yeast infection (Caused by Cryptococcus neoformans and Histoplasma capsulatum), and dental diseases due to Vitamin E.[7-18] Disulfiram is a cream-white, almost odourless, slightly bitter crystalline powder practically insoluble in water.

LITERATURE REVIEW

Sebert *et al.* **(1976)** prepared tablets containing 79% Disulfiram by direct compression. Optimum tablet properties were achieved with 14% Microcrystalline Cellulose (Avicel PH 102), 5% Sodium Carboxy-methyl Starch (Primojel) and 1% of both Talc and Magnesium Stearate.

Jean- Luc Dubois *et al.* **(1985)** investigated the dissolution rates ($mg.min^{-1}$) of 10 drugs, solid dispersed by fusion in polyethylene glycol 6000 (PEG 6000) by rotating disc methodology. During fusion, chain scission of the PEG 6000 occurred in the presence of several drugs. PEG 6000 was found incompatible with Disulfiram, Furosemide, Chlorothiazide and Chlorpropamide.

Anderson *et al.* **(1992)** investigated the comparison of the bioavailability of Disulfiram (DSF) after administration of non-effervescent Antabuse® tablets (CP Pharmaceuticals, UK) and Antabuse® effervescent tablets Antabuse® (A/S Dumex, DK) in two cross-over studies. The bioavailability of DSF after administration of non-effervescent was found to be only 27 % of that achieved with effervescent tablets. The relative bioavailability of DSF after administration of non-effervescent compared with effervescent tablets was found to be only 34%. Thus, the bioavailability of DSF appears to depend on both the formulation (preparation) and the mode of administration. A lack of bioequivalence between the two investigated DSF preparations was found.

Advertorial case study-GEA Courtoy's Performa P **(2008)** has reported difficult formulations on conventional presses include Ibuprofen – 400 mg, Paracetamol – 500 mg and Disulfiram – 500 mg. The formulation composition includes, Disulfiram – 71%, Lactose 200 as diluent and Starch as binder and disintegrant. The process followed was Starch paste granulation in a Rapid Mixer Granulator / High Shear Mixer Granulator followed by drying in Fluid Bed Dryer. The final blend was compressed at a maximum speed of 50,000 Tablets per hour to prevent capping of tablets. The compression is done with an extended dwell time.

Naveen Pathak *et al.* **(2011)** investigated the preparation of Disulfiram Tablets by Dry granulation technique. Poor compressibility, static charge and poor flow of blend were observed during formulation. The finalized formulation includes, Disulfiram USP – 500 mg, Colloidal Silicon Dioxide (Aerosil 200) – 20 mg, Lactose Anhydrous (Supertab 21 AN) – 154 mg, Microcrystalline Cellulose (Avicel PH 112) – 60 mg, Sodium Starch Glycolate Type

A (Glycolys) – 14 mg, Stearic Acid (Speziol L2SM GF) – 6 mg and Magnesium Stearate - 6 mg with a total tablet weight of 760 mg.

Nisrina Ramadhani *et al.* **(2014)** demonstrated the development and characterisation of solid dispersion oral tablets, containing the poorly water-soluble drug Disulfiram, prepared using both the hot melt and solvent evaporation methods and manufactured from two different polymers, Kolliphor® P 188 and P 237, specifically designed for the manufacture of solid dispersions. The Disulfiram in the Kolliphor® P 188 solvent evaporated and 60 °C hot melt tablets retained 50.5 and 44.1% of its crystallinity, while the Disulfiram in the 80 °C hot melt tablets was completely amorphous. Whereas the Disulfiram in the Kolliphor® P 237 solvent evaporated tablets retained 45.2% crystallinity, while the Disulfiram in both of the hot melt tablets was completely in its amorphous form. This paper demonstrates that the Disulfiram solid dispersions tablets have an enhanced release rate of Disulfiram compared to the control tablets.

Patent Number: US 2014/0275242 A1; Title: Hot Melt Granulation Formulations of Poorly Water-Soluble Active Agents; Inventors: Chaoju Xiao and Boyong Li; Assignee: Mylan Laboratories Inc, USA; Filed Date: March 14, 2014; Expiry Date: March 14, 2034; **Abstract:** In this patent, Hot melt extrusion process was used to manufacture the drug – excipient granules for compression to overcome the Picking, Sticking, Poor Compressibility and Poor solubility of Disulfiram. The temperature for hot melt extrusion was at a temperature around 85°C (85°C-90°C). The unit composition includes Disulfiram – 52.63 mg, Stearic Acid – 4.21 mg, Microcrystalline Cellulose – 18.42 mg, Lactose Anhydrous – 18.42, Sodium Starch Glycolate – 5.57 mg, Colloidal Silicon Dioxide – 0.50 mg and Magnesium Stearate – 0.25 mg with a total tablet weight of 100 mg.

NOVELTY

Based on the literature information, Dry granulation / hot melt extrusion granulation / direct compression process will not be explored due to stability and feasibility. In view of environment friendly and cost effectiveness into consideration, No non-aqueous solvent will be used. Disulfiram Tablets will be manufactured by Wet granulation process. Water will be used for preparing the binder solution. The formulation will be fully optimized with respect to composition and process to ensure good blend flow and tableting without defects. Addition of solubilizers / surfactants will be avoided instead will focus on the effect of API particle

size especially milling and micronization influence on dissolution against the internationally marketed product will be evaluated.

OBJECTIVE

The objective of the research is to make Disulfiram Tablets which will exhibit a better mechanical strength and morphology as compared to the internationally marketed product. Disulfiram being a highly variable drug, this research will also focus on the influence of milling and micronization on bioavailability of the drug product and accordingly particle size specification of API will be finalized.

RESEARCH PLAN

Disulfiram API exhibits poor flow and compressibility. Hence it was decided to formulate the Disulfiram tablets by aqueous wet granulation method. Hydroxy propylcellulose (Klucel LF) as binder; Silicified Microcrystalline Cellulose (Prosolv SMCC HD 90) as diluents; Sodium Starch Glycolate Type A (Primojel) as disintegrant; Colloidal Silicon Dioxide (Aerosil 200) as glidant and antistatic agent; Stearic Acid (Stellipress 1200 Poudre) and Non Bovine Grade Magnesium Stearate (Tablube) as lubricants were selected.

MATERIALS AND METHODS

Excipients & Reagents

Disulfiram was obtained from Farchemia; Prosolv SMCC HD 90 from JRS Pharma; Primojel from DFE Pharma; Klucel LF from Ashland Inc; Tablube from Nitika Chemicals; Aerosil 200 from Evonik; Stellipress 1200 Poudre from Stearinerie Dubois; Stepanol WA100 from Stephane; Ethanol and Methanol from Avantor Performance Materials; Potassium Dihydrogen Phosphate and Potassium Hydroxide from Merck Specialities Ltd.

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 0.25 mm screen of Cadmach Machinery; 40°C / 75% RH Stability Chamber of Thermolab Scientific Equiments; HPLC of Waters; Vibrosifter of Gansons Engineering; 12.8 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 15 minutes. Dissolution is performed in 900 mL Purified Water with 2% Sodium Lauryl Sulphate. The parameters include, USP-II (Paddle), 100 RPM. Time Points: 15, 30, 45, 60, 75, 90, 105 and 120 minutes. λmax at 254 nm. Specification: NLT 75 % (Q) of the labeled amount in 120 minutes.

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

Disulfiram 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

Disulfiram Tablets 500 mg manufactured by Duramed Pharmaceuticals Inc. was characterized with respect to Inactive ingredients details, Weight, Thickness, Diameter, Disintegration Time and Dissolution Profile and Packaging configuration.

API Characterization

Disulfiram (both milled and jet mill micronized) 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

Divide the Hydroxypropyl Cellulose (Klucel LF) required for the batch into two parts, 70% of the total batch quantity of Hydroxypropyl Cellulose (Klucel LF) is dissolved in terms of 12% w/w solids in Purified Water as a binder solution. The remaining 30% of the total batch quantity of Hydroxypropyl Cellulose (Klucel LF) is sifted along with Disulfiram (milled through Comminuting mill equipped with 0.25 mm screen at fast speed impact forward configuration) and Sodium Starch Glycolate (Primojel) through # 20 ASTM sieve. 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 binder solution of Hydroxypropyl Cellulose (Klucel LF) at high impeller and chopper speed. The binder solution container was rinsed with purified water which is 12% of the total quantity of binder solution prepared and rinsings were added to the contents of High Shear Mixer Granulator. The wet granules were dried in Fluid Bed Dryer Processor & Granulator at 50°C till LOD is not less than or equal to 1%. The dried granules were milled through Multi Mill equipped with 1.5 mm screen at fast speed knife forward configuration. The milled granules were blended in Double Cone Blender at 15 RPM for 5 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 15 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 12.8 mm Round Shaped Standard Concave Punch Tooling. The details of Formulation Prototypes are shown in Table 1. The drying parameters include, Inlet Air Temperature – 50° ± 5°C; Exhaust Temperature – 40° ± 5°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. 100 Tablets were packed in 150 cc HDPE bottle with 38 mm Child Resistant Cap with cotton as dunnage 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), 100 RPM, 900 mL, Purified Water with 2% Sodium Lauryl Sulphate @ 37 ± 0.5°C. Time Points: 15, 30, 45, 60, 75, 90, 105 and 120 minutes. Specification: NLT 75 % (Q) of the labeled amount in 120 minutes.

In vivo Study

An open-label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, crossover, bioequivalence study of Disulfiram Tablets, 500 mg (made with micronized API – Test 1), Disulfiram Tablets, 500 mg (made with milled API - Test 2), with that of internationally marketed product was conducted in 15 healthy adult human subjects under fasting 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 to the subjects and 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. The pre-dose 0 hour blood sample was collected before dosing and post dose samples were collected at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0 and 72.0 hours. Subjects were provided standard diet and continuously monitored for well-being and safety throughout the study. The concentration of S-methyl N, N-diethyldithiocarbamate (Me-DDC), which is an active metabolite of Disulfiram 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 above 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 tests and reference products were calculated by using SAS® statistical software version 9.1.3 from SAS Institute Inc, USA. For Disulfiram, in terms of S-methyl N,N-diethyldithiocarbamate (Me-DDC), which is an active metabolite of Disulfiram, the 90% confidence interval of the relative mean Cmax, AUC0-t and AUC0-∞ of the tests 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	**500 mg**
Description	White round, scored tablets debossed OP 706 on one side and plain on the other side.
Excipients	Colloidal Silicon Dioxide, Anhydrous Lactose, Magnesium Sterarate, Microcrystalline Cellulose, Sodium Starch Glycolate and Stearic Acid
Diameter	12.76 mm Round Standard Concave
Avg Weight(mg)	781.4
Avg Thickness (mm)	6.15
Avg Hardness (kP)	12.0
% LOD (105°C / Automode)	1.17%
Disintegration Time	1 min 55 secs
Time (min)	**Dissolution (mean % drug dissolved) n=6 units**
15	51.6
30	62.5
45	70.8

60	74.4
75	77.5
90	79.9
105	81.4
120	83.3
Pack Details	100 Tablets in 100 cc HDPE Bottle with cotton coils as dunnage capped with 38 mm Polypropylene Child Resistant Cap with induction seal liner

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	White to off-white crystalline powder
Bulk Density	0.51 g / ml
Tapped Density	0.77 g / ml
Compressibility Index	34%
Hausner Ratio	1.51
Repose Angle	39.3°
Particle Size Distribution	
By Malvern Mastersizer®	
For API from Manufacturer	d10 20.48 microns, d50 60.93 microns & d90 171.07 microns
For Milled API	d10 12.47 microns, d50 42.83 microns & d90 83.33 microns
For Micronized API	d10 2.33 microns, d50 6.57 microns & d90 12.38 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**
Disulfiram	**%ASSAY**	99.94	100.61	103.77
	%TI	0.054	0.044	0.045
	%Moisture	0.2	0.1	0.0
Disulfiram+ Hydroxypropyl Cellulose (1:1)	**%ASSAY**	97.81	95.98	100.67
	%TI	0.057	0.052	0.045
	%Moisture	1.32	1.21	1.6
Disulfiram+ Lactose Anhydrous (1:1)	**%ASSAY**	88.63	89.06	89.45
	%TI	0.050	0.047	0.035

	%Moisture	2.16	2.23	2.01
Disulfiram+ Crospovidone (1:1)	**%ASSAY**	92.76	92.99	94.44
	%TI	0.054	0.047	0.047
	%Moisture	1.6	1.54	1.75
Disulfiram+ Microcrystalline Cellulose (1:1)	**%ASSAY**	93.42	96.07	95.17
	%TI	0.058	0.040	0.042
	%Moisture	0.83	0.92	0.74
Disulfiram+Sodium Starch Glycolate (1:1)	**%ASSAY**	99.88	100.43	99.33
	%TI	0.052	0.047	0.040
	%Moisture	1.99	1.86	2.02
Disulfiram+ Croscarmellose Sodium(1:1)	**%ASSAY**	95.08	97.18	97.93
	%TI	0.054	0.041	0.047
	%Moisture	3.8	2.6	3.88
Disulfiram+Pregelatinized Starch (1:1)	**%ASSAY**	84.37	83.26	83.65
	%TI	0.048	0.046	0.038
	%Moisture	2.03	2.03	3.37
Disulfiram+ Microcrystalline Cellulose (1:1)	**%ASSAY**	102.25	97.85	101.75
	%TI	0.057	0.034	0.048
	%Moisture	0.25	0.26	0.38
Disulfiram+Colloidal Silicon Dioxide (1:1)	**%ASSAY**	101.70	102.91	104.96
	%TI	0.054	0.064	0.047
	%Moisture	0.19	0.14	0.51
Disulfiram+Magnesium Stearate (1:1)	**%ASSAY**	100.87	98.07	101.53
	%TI	0.052	0.041	0.053
	%Moisture	0.24	0.29	0.38
Disulfiram+Stearic Acid (1:1)	**%ASSAY**	96.06	95.76	96.30
	%TI	0.055	0.040	0.045
	%Moisture	0.22	0.11	0.19
Disulfiram+Sodium Stearyl Fumarate (1:1)	**%ASSAY**	99.02	100.23	99.85
	%TI	0.060	0.045	0.048
	%Moisture	0.12	0.0	0.0
Disulfiram+ Ultramicronized Talc (1:1)	**%ASSAY**	101.22	102.85	101.80
	%TI	0.054	0.044	0.043
	%Moisture	0.26	0.25	0.23

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

Formulation Process And Characterization

The composition details were elaborated in Table 4 and the process details with characterization of blend and tablets were provided in Table 5. From the details it is evident that the composition and process is robust enough to produce the drug product with good physico-chemical attributes. Dissolution profile of the prepared tablets of Disulfiram was compared against the internationally marketed product, of Disulfiram) Tablets, 500 mg. Both the test products complied to the dissolution specification of not less than 75% (Q) in 120 minutes. See Table 6 and Figures 1 for further details.

Table 4. Formulation.

S.No	Ingredients	A	B
		mg / tablet	
Intragranular Ingredients			
1	Disulfiram (Milled)	500.00	-
2	Disulfiram (Micronized)	-	500.00
3	Sodium Starch Glycolate Type A (Primojel)	35.00	35.00
4	Hydroxypropyl Cellulose (Klucel LF)	20.00	20.00
5	Purified Water	QS	QS
Total Intragranular Ingredients		555.00	555.00
Extragranular Ingredients			
6	Silicified Microcrystalline Cellulose (Prosolv SMCC HD 90)	99.00	99.00
7	Sodium Starch Glycolate Type A (Primojel)	16.00	16.00
8	Colloidal Silicon Dioxide (Aerosil 200)	16.00	16.00
9	Stearic Acid (Stellipress 1200 Poudre)	12.00	12.00
10	Magnesium Stearate (Tablube)	2.00	2.00
Total Extragranular Ingredients		145.00	145.00
Total Tablet Weight		700.00	700.00

Table 5. Blend and Tablet Characterization.

Particulars	A	B
Dry Mix Time(min)	15	15
Dry Mix LOD (%)	0.92	0.62
Dry Mix Bulk Density (g / ml)	0.14	0.14
% Granulation Binder Uptake	42	42
Kneading Time (min)	3	3
Total Granulation Time (min)	24	24
Drying Time in FBP (min)	18	22
LOD of Dried Granules (%)	0.64	0.94
Prelubrication Blending (min)	15	15
Lubrication Blending (min)	5	5
Bulk Density (g/ml)	0.54	0.52
Tapped Density (g/ml)	0.74	0.70
Compressibility Index (%)	27.0	25.4
Hausner Ratio	1.37	1.34
LOD of Final Blend (%)	1.32	1.28
Repose Angle (°)	29.6	29.4
Turret Speed (RPM)	20	20
Average Weight (mg)	701.3	701.1
Average Thickness (mm)	6.05	6.04
Average Hardness (kP)	9-21	9-21
Friability (%)	0.38	0.25
Disintegration Time (mins)	2.20	3.15
Picking / Sticking /Striation	×	×
Sieve # (ASTM)	**Final Blend PSD (% Retained)**	
20	2	10

40	18	25
60	22	7.5
80	12	5
100	8	7.5
140	12	10
200	10	15
Pan	16	20

Table 6. Dissolution Profile Comparison of Test Products Vs Marketed Product.

Particulars	**Mean % Dissolved (n=6 units)**							
	Time (Minutes)							
	15	**30**	**45**	**60**	**75**	**90**	**105**	**120**
BRAND	51.6	62.54	70.8	74.4	77.5	79.9	81.4	83.3
Batch A (API Milled)	51.3	77.6	88.3	92.3	96.5	98.3	100.0	100.4
Batch B (API Micronized)	66.5	83.8	91.11	95	97.3	98.5	99.6	100.1
Medium**:** 900 ml, Purified water @ 37 ± 0.5ºC, USP-II (Paddle), 100 RPM, Specification: NLT 75% (Q) in 120 minutes								

Stability Study

The prepared Disulfiram tablets of Batch A (Milled API) and Batch B (Micronized API) were packed in 100 tablets per 150 cc HDPE bottle and capped with 38 mm child resistant polypropylene closure with Cotton coil as dunnage 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 7 for further details.

In vivo Study

In vivo study was carried out for test formulations, Batch A (Milled API) and Batch B (Mirconized API) and the internationally marketed product. The plasma levels of Disulfiram were determined. The mean concentration-time profiles for the marketed and test products of Disulfiram under fasted condition were shown in Table 8 and Figure 2 respectively. T/R ratio shows that the test product made with milled API is bioequivalent to the internationally marketed formulation. The power of the study and ISCV can be further improved by increasing the subjects / volunteers.

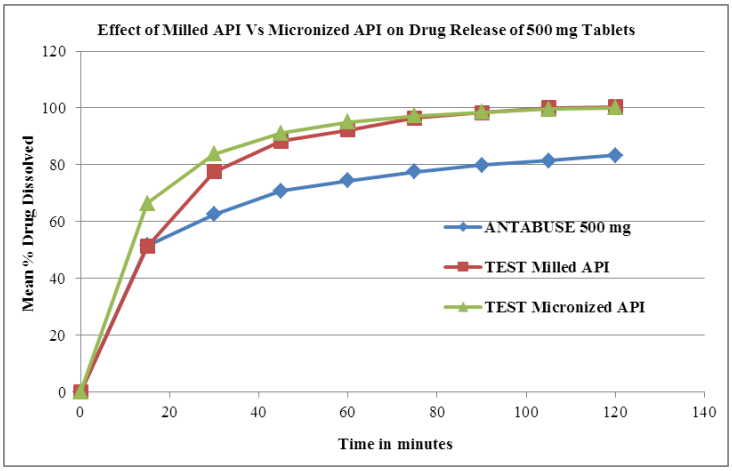

Figure 1. Dissolution Profile Comparison.

Table 7. Stability Results at 40°C ± 2°C / 75 ± 5% RH.

Test	Specification	Initial	1st Month	2nd Month	3rd Month
Batch A					
Description	**	Comply	Comply	Comply	Comply
Disintegration Time (min)	NMT 15 minutes	3.26±0.59	3.38±0.61	3.24±0.32	3.29±0.47
Dissolution[%mean]	NLT 75%(Q) in 120 minutes	100.4±0.50	99.1±0.34	100.2±0.41	98.7±0.51
Assay (%)	90-110	99.9	99.1	98.1	99.4
Related Substances (%)					
Highest Unknown Impurity	NMT 0.24	0.044	0.027	0.053	0.072
Total Impurities	NMT 1.0	0.108	0.076	0.111	0.126
Batch B					
Description	**	Comply	Comply	Comply	Comply
Disintegration Time (min)	NMT 15 minutes	3.15±1.83	3.29±1.68	3.11±1.52	3.27±1.71
Dissolution	NLT 75%(Q) in 120 minutes	100.1±0.60	98.7±0.43	99.8±0.46	100.1±0.72
Assay (%)	90-110	100.9	99.8	101.0	99.6
Related Substances (%)					
Highest Unknown Impurity	NMT 0.24	0.044	0.027	0.044	0.095
Total Impurities	NMT 1.0	0.113	0.062	0.102	0.152

**White to off-white colored, uncoated, round shaped biconvex tablets; on stability no morphological change observed.

Table 8 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 – 1 (Micronized API)				
Arithmetic Mean	80.7731	957.5747	1007.1244	2.50
Geometric LS Mean	4.3423	6.8016	6.8524	
Standard Deviation	28.2446	342.9588	358.2954	
Test Product – 2 (Milled API)				
Arithmetic Mean	56.9497	772.9077	807.9112	4.50
Geometric LS Mean	3.9738	6.5692	6.6168	
Standard Deviation	21.7416	283.3861	289.4438	
Reference Product				
Arithmetic Mean	48.8440	714.0690	748.7907	4.50
Geometric LS Mean	3.7646	6.4913	6.5357	
Standard Deviation	30.8236	326.3148	351.0084	
T / R Ratio				
T1/R (%)	178.20	136.39	137.26	-
T2/R (%)	123.27	108.10	108.45	
90% Confidence Interval (T1/R) (Micronized API)				
Lower Limit (%)	155.89	119.18	120.08	-
Upper Limit (%)	203.71	156.08	156.91	
90% Confidence Interval (T2/R) (Milled API)				
Lower Limit (%)	107.84	94.46	94.87	-
Upper Limit (%)	140.92	123.71	123.97	
ISCV (%)				
-	21.73	21.91	21.73	-

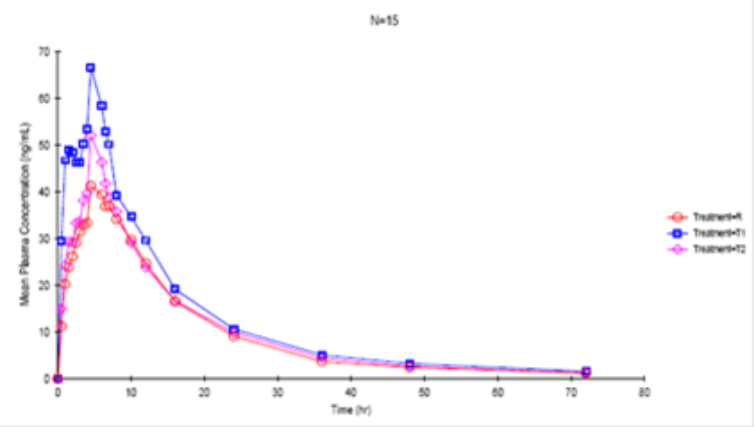

Figure. 2: Mean Concentration Time Profile – Fasting Study.

CONCLUSION

Disulfiram tablets were successfully formulated by a simple aqueous wet granulation process. Disulfiram is an insoluble and highly biovariable drug. Tablet morphology, mechanical strength, disintegration time and dissolution were fixed as prerequisite design attributes. Formulation was done using both milled and micronized API. Hydroxypropyl cellulose

(Klucel LF) was selected as binder. Silicified Microcrystalline Cellulose (Prosolv SMCC HD 90) was selected as diluent. Sodium Starch Glycolate Type A (Primojel) was selected as disintegrant. Colloidal Silicon Dioxide (Aerosil 200) was selected as glidant and antistatic agent. Stearic Acid (Stellipress 1200 Poudre) and Non Bovine Grade Magnesium Stearate (Tablube) were selected as lubricants. Disulfiram tablets made with both milled and micronized API showed comparable disintegration and the dissolution profile to the internationally marketed product. Disulfiram tablets made with both milled and micronized API were subjected to accelerated stability study in HDPE bottle pack and the physico-chemical properties were found to be stable. The prepared Disulfiram tablets made with both milled and micronized API was subjected to *in vivo* bioequivalence study under fast condition against the internationally marketed product. Disulfiram Tablets made with milled API showed promising bioequivalence with that of the marketed product.

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