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Development, Evaluation and Stability Studies of Zidovudine and Lamivudine (ZILA) Tablet Dosage Form

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ABSTRACT

Tablets containing zidovudine and lamivudine (ZILA) were prepared by direct compression method. Optimization studies were done for the selection of glidant, lubricant and coating materials. Evaluation of granuleswere done on the basis of preformulation studies. The prepared tablets were evaluated for physicochemical properties. The in- vitro release studies were performed as per USP and compared with marketed product. The release of zidovudine and lamivudine were analysed by high performance liquid chromatography (HPLC). The ZILA tablets exhibited better release characteristics than the marketed product. Stabilities studies were performed in both blister as well as cold form blister packings. Stabilities studies revealed the suitability of blister package in comparison to the cold form blister packing. From the study it was concluded that the selected composition can be used for the preparation of tablets that can be used for the treatment of HIV-1 and Hepatitis-B after performing studies on animals for its suitability and efficacy.

INTRODUCTION

Tablets are solid dosage forms containing medicinal substances with or without suitable diluents. Tablets are simple and convenient to use. They provide an accurately measured dosage of the active ingredient in a convenient portable package, and can be designed to protect unstable medications or disguise unpalatable ingredients. They may be classified according to the method of manufacture either compressed tablets or molded tablets. The compressed tablets are the most widely used dosage form in world and the vast majority of all tablets manufactured are made by compression. The compressed tablet is the most popular dosage form in use nowadays. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of. these are compressed tablets. The current course of therapy for treating HIV infection consists of use of combination of at least two drugs to avoid/delay the development of resistance by the human immunodeficiency virus (HIV). Multi-drug therapy has become the standard treatment for acquired immunodeficiency syndrome (AIDS) (Clercq, 2002). It is also desired to minimize potential dose dependent side effect (Beach, 1998). The combination of the two drugs has a stronger and more sustained effect than using either drug alone, and assists in reducing pill burden and in aiding compliance with the antiretroviral drug therapy. Lamivudine and zidovudine are used for the treatment of infections with the human immunodeficiency virus (HIV) in patients with or without acquired immunodeficiency syndrome (AIDS) (Chancellor *et al*., 1997). Both are in a class of drugs called nucleoside analog reverse-transcriptase inhibitor (NRTI). They inhibit the activity of reverse transcriptase and block the production of DNA and new

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viruses (Saavedra-Lozano *et al*., 2004). The HIV virus develops resistance to the effects of the individual drugs when used alone either lamivudine or zidovudine. It is more difficult for the HIV virus to develop resistance for the drugs when combined together (lamivudine and zidovudine) since it must develop resistance to both drugs. Thus, the combined therapy is considered to be more effective than lamivudine or zidovudine alone. AIDS treatment requires new therapeutic strategy including the combination of these antiretroviral drugs. The introduction of highly effective combination regimens of drugs has led to substantial improvements in morbidity and mortality. It reduces the viral load in the body and raises CD4 cell count (Eron *et al*., 1995). The aim of the present study was to develop, evaluate ZILA tablets and compare with the marketed one. The marketed brand was selected from Glaxosmithkline, USA.

MATERIAL AND METHODS

Preparation of Tablet

The dry granulation process was used for the preparation of tablet containing Zidovudine and Lamivudine (ZILA). The optimized amount of ingredients required for the preparation of each tablet is given in Table 1. Accurately weighed ingredients were sifted through 60, 80 & 20 mesh sieve. All the ingredients except magnesium stearate were blended for 30 min using an octagonal blender (Kalweka VDM, Gujrat, India). Now magnesium stearate was added and lubricated for 10 minutes. Fines below 60 mesh (%), bulk density, tapped density and loss on drying of lubricated blend were calculated. The granules were compressed into tablets using compression machine (Cadmach Machinery Co. Pvt. Ltd., Ahmedabad, India). Temperature below 25^{o} C and relative humidity between 45-55 % RH was maintained throughout the manufacturing process (Agashe & Jain, 2001).

Table. 1: Optimized formula for the preparation and coating ZILA tablets.

Ingredients	*Quantity (mg/tablet)*
Lamivudine	300.0
Zidovudine	150.0
Microcrystalline Cellulose USP	254.0
Sodium Starch Glycolate	29.0
Colloidal Silicon Dioxide	2.9
Magnesium Stearate USP	14.5

Selection of glidant

Glidants are used to promote powder flow by reducing interparticle friction and cohesion. Different glidants including silica, fumed silica, magnesium carbonate/60, magnesium carbonate/40, magnesium carbonate/20, magnesium carbonate, talc and colloidal silicon dioxide were tried and included in the formula of ZILA tablets for the selection of suitable one (Table 2).

Selection of lubricant

Lubricants are agents added in small quantities to tablet formulations to decrease friction at the interface between a tablet's surface and the die wall during ejection and reduce wear on punches & dies. Different lubricants including magnesium stearate USP, zinc stearate USP, stearic acid USP were included in the formula to select the suitable one (Table 3). The most suitable lubricant was selected based on the properties of granules including bulk density, tapped bulk density, Carr's index, Hausner's ratio and angle of repose (Subharamanyam, 2002; Carr, 1965; Banker & Anderson, 1986).

Table. 2: Manufacturing formula for the selection of suitable glidant

Ingredients	Formula							
	F1	F2	F3	F4	F5	F6	F7	F8
Lamivudine	150	150	150	150	150	150	150	150
Zidovudine	300	300	300	300	300	300	300	300
Microcrystalline Cellulose USP	254	254	254	254	254	254	254	254
Sodium Starch Glycolate	29	29	29	29	29	29	29	29
Magnesium Stearate USP	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Silica	2.9	--	--	--	--	--	--	--
Fumed silica	--	2.9	--	--	--	--	--	--
Magnesium carbonate/60	--	--	2.9	--	--	--	--	--
Magnesium carbonate/40	--	--	--	2.9	--	--	--	--
Magnesium carbonate/20	--	--	--	--	2.9	--	--	--
Magnesium carbonate	--	--	--	--	--	2.9	--	--
Talc	--	--	--	--	--	--	2.9	--
Colloidal Silicon Dioxide	--	--	--	--	--	--	--	2.9
Total weight (mg)	750	750	750	750	750	750	750	750

Table. 3: Manufacturing formula for lubricant selection.

Ingredients	Formula		
	F9	F10	F11
Lamivudine	150	150	150
Zidovudine	300	300	300
Microcrystalline Cellulose	254	254	254
Sodium Starch Glycolate	29	29	29
Colloidal Silicon Dioxide	2.9	2.9	2.9
Magnesium Stearate USP	14.5	--	--
Zinc Stearate USP	--	14.5	--
Stearic acid USP	--	--	14.5
Total weight (mg)	750	750	750

EVALUATION

Evaluation of granules

Bulk Density

The granules were passed through #60BSS and collected on a piece of paper. Accurately weighed quantity of granules (25 g) was transferred in 50 ml graduated cylinder. Powder was carefully levelled without compacting, and read the unsettled apparent volume (V0). Apparent bulk density in gm/ml was calculated by the following formula:

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Bulk volume}}$$

Tapped density

The previously sifted granules through #60BSS were accurately weighed (25 g) and transferred in a graduated cylinder (50 ml). The cylinder containing sample was mechanically tapped by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester (ETD 1020, Electrolab, Mumbai, India) that provides a fixed drop of 14± 2 mm at a nominal rate of 300 drops per minute.

Initially the cylinder was tapped for 500 times and the tapped volume (V1) was measured to the nearest graduated units. The taping was repeated for an additional 750 times and tapped volume (V2) was measured to the nearest graduated units. The final volume (V2) was taken in case the difference between the two volumes was found to be less than 2% (w/w). The tapped bulk density (gm/ml) was calculated using the following formula:

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume}}$$

Carr's Index

The Compressibility Index of the powder blend was determined by Carr's compressibility index. It depends upon the BD and TD of a powder and evaluates the rate at which it packed down. The following formula was used for the determination of Carr's Index:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's Ratio

The Hausner's ratio is a number that is correlated to the flow ability of a powder or granular material. Hausner's ratio was determined using the following formula:

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose

The angle of repose of powder was determined by the funnel method. The accurately weighed powder blend was taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend (Train, 1958). The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation:

$$\text{Ø} = \tan^{-1} h/r$$

Where, h and r are the height and radius of the powder cone respectively.

1.

2. **Evaluation of Tablets**

The manufactured tablets were evaluated for description, thickness, hardness, friability, weight variation, disintegration time and dissolution test (Gohel *et al*., 2005; Patel & Baria 2009).

Dissolution study

Dissolution study was carried out in official compendia by using USP II (Paddle type) dissolution apparatus (USP, XXXIV, Rockville, MD, 2004). The dissolution media containing 0.1 M HCl (pH 1.2) was taken in each vessel of the apparatus. The volume of compendia were used 900 mL and the temperature of the media was regulated at 37° C ± 0.5°C (Tavakoli *et al*., 2012; Guidance for Industry: FDA, CDER, Rockville, MD, 1995). Immediately one tablet was transferred into each vessel of the apparatus. The paddle was rotated at a speed of 75 rpm. The distance between the inside bottom of the vessel and the paddle was maintained at 2.5 cm during the test. The study was performed for a period of 30 min and the sampling was done at 10, 15, 20 and 30 minutes. Fresh media was replaced after each sampling and analyzed for UV absorbance by HPLC at λ_{max}. Samples were analyzed to estimate the release of the drug (ZILA).

Accelerated Stability study

Optimized batch of F8 was packed in blister pack and was placed for stability studies at 25˚C±2˚C/65%RH±5% RH and 40˚C±2˚C /75% RH±5%RH for 2 months. Samples were collected at every month and evaluated for description, water content and assay of zidovudine and lamivudine to determine the effect of storage conditions (FDA: guidelines for stability studies, www.ich.org/ stability-testing-for-new-dosage-forms.htm.). The water content was determined by Karl Fischer titration method (Mendham *et al*., 2000).

Hplc Method of Analysis

The released ZILA from each tablet was analyzed by high performance liquid chromatography (HPLC) (Agilent, USA). HPLC was equipped with quaternary G1311A pumps, variable wavelength programmable UV-Vis detector. The HPLC column with a reverse phase C18, 25cm, 4.6mm, 5µm Column (Intersil ODS 3v is suitable) and software Chromeleon 6.8 were used. The whole system was kept at ambient conditions. The mobile phase was acetate buffer (pH 4.5)/methanol (1:1) with a flow rate of 1.0 ml/min. The injection volume was 10 µl and the elute was analysed at 270 nm.

Table. 4: Result of blend parameters of formulations.

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner's Ratio	Angle of repose (degrees)
F1	0.56	0.71	21.1	1.26	36.0
F2	0.50	0.70	28.8	1.40	36.6
F3	0.53	0.74	28.3	1.39	31.3
F4	0.51	0.68	25	1.33	35.6
F5	0.57	0.71	19.7	1.24	36.5
F6	0.59	0.75	21.3	1.27	36.6
F7	0.63	0.75	16	1.19	38.1
F8	0.63	0.74	14.8	1.17	30.6

Table. 5: Result of post-compression parameters of formulations.

Formulation	Weight (mg)	Hardness (Newton)	Thickness (mm)	Friability (%)	Disintegration Time (Min.)
F1	0.765	344	3.70	0.400	5
F2	0.763	340	3.68	0.286	4.5
F3	0.767	345	3.69	0.467	4.8
F4	0.759	345	3.66	0.466	5
F5	0.759	325	3.21	0.525	5
F6	0.760	336	3.69	0.458	5.2
F7	0.765	324	3.67	0.393	5.5
F8	0.756	318	3.60	0.520	5

Table. 6: Result of blend parameters of lubricant selection formulation.

Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner's ratio	Angle of repose (degree)
F9	0.63	0.74	14.8	1.17	30.6
F10	0.63	0.75	16	1.19	38.1
F11	0.59	0.75	21.3	1.27	36.6

Table. 7: Post-compression result of suitable lubricant selection formulation.

Formulation	Weight (mg)	Hardness (KP)	Thickness (mm)	Friability (%)	Disintegration Time (Min.)
F9	0.756	318	3.69	0.458	5
F10	0.765	324	3.67	0.393	5
F11	0.760	336	3.60	0.520	5

Table. 8: Stability Studies of Formulation F8.

Cold Form Blister (25°C± 2°C/ 65% ± 5% RH)				
Months	Description	Water content (%) (w/w)	Assay	
			LAMIVUDINE	ZIDOVUDINE
1	White	0.091	97.51	100.41
2	White	0.039	95.53	98.34
		Cold Form Blister (40°C ± 2°C/ 75% ± 5% RH)		
1	White	0.089	95.89	96.81
2	White	0.051	93.72	95.37
		Blister (25°C ± 2°C/ 65% ± 5% RH)		
1	White	0.088	99.67	101.75
2	White	0.041	97.99	99.62
		Blister (40°C ± 2°C/ 75% ± 5% RH)		
1	White	0.090	98.98	99.58
2	White	0.043	95.13	98.74

RESULTS AND DISCUSSION

After preformulation studies it was found that dry granulation method was selected for the ZILA tablet formulation. The dry granulation process is used to form granules without using a liquid solution because the product to be granulated may be sensitive to moisture and heat. Forming granules without moisture requires compacting and densifying the powders. Dry granulation can be conducted on a tablet press using slugging tooling or on a roller compactor commonly referred to as a chilsonator.

Selection of glidants

Granules were prepared containing different types of glidants and evaluated for different parameters including bulk density (g/ml), tapped density (g/ml), Carr's Index (%), Hausner's Ratio, angle of repose (degrees) as shown in table 4. These granules were compressed into tablets and further evaluated for the parameters including weight, hardness, thickness, friability, and disintegration time (Table 5).

The powder flow of the granules was found to be very poor in case of F1, F2, F3, F5, F6 and F7 formulations. Moreover the hardness of compressed tablets was found to be very less as compared to innovator and failed the friability test in case of F1, F2 and F3 respectively. The pre-compression parameters are satisfactory but post-compression parameters were not satisfactory for F4 formulation. The formulation F8 was taken for further study on the basis of satisfactory results of pre-compression as well as

post-compression parameters. Thus, the formulation F8 was selected for further studies including lubricant selection and dissolution studies.

Selection of lubricant

The tablet of composition of formulation F8 was further evaluated for the selection of suitable lubricant. Different lubricants (e.g., magnesium stearate USP, zinc stearate USP, stearic acid USP) were added in F9, F10 and F11 formulations. Granules were prepared and evaluated for blend parameters including bulk density, tapped bulk density, Carr's index, Hausner's ratio and angle of repose as shown in table 6. These granules were further compressed into tablets and evaluated for weight (mg), hardness (KP), thickness (mm), friability (%), and disintegration time (Min.) as shown in table 7. Based on the blend parameter and post-compression results for lubricant selection, magnesium stearate was found to be better and suitable lubricant for the preparation of ZILA tablets.

Dissolution Studies

The dissolution studies of ZILA tablets were performed and evaluated. The cumulative amount of drug released (%) at different time intervals was estimated for ZILA tablets of different composition and the innovator tablet (Figure 1). The composition of ZILA tablets (F1 to F8) was same and magnesium stearate was included as lubricant with each formula of tablets. All the tablets were found to release more than 90% after 30 min. Maximum release was found to be 100.2 % by F8 formulation. However, the innovator tablet was found to release a maximum of 98.2% of drug. Thus, the release (%) of formulation F8 was better than the innovator.

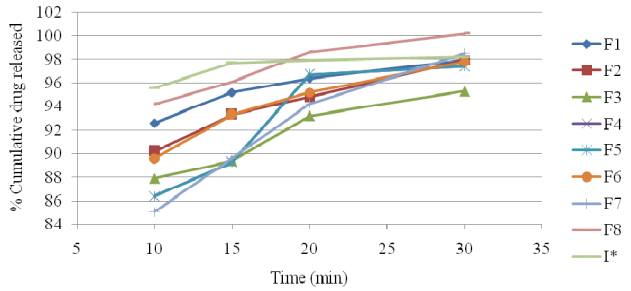

Fig 1: Comparison of in-vitro dissolution studies of different formulations and innovator tablet.

Accelerated stability studies

The selected formulation F8 was evaluated after two months of the stability period. Tablets were evaluated at different temperature and humidity conditions and the result are mentioned in table 8. It was found that the lamivudine, zidovudine tablets are more stable in the blister pack at the both of the temperature and relative humidity of stability period.

CONCLUSION

The present investigation was carried out to develop a tablet dosage form of lamivudine and zidovudine. Dry granulation method was found to be most suitable method for formulation of low dose dosage form in comparison of wet granulation. Colloidal silicon dioxide was found to be suitable glidant for the present tablet dosage form and showed better palatability. Magnesium stearate was found to be the suitable lubricant for the study. The tablet dosage forms were evaluated for blend characteristics and compression parameters. The dissolution studies revealed that the formulation F8 showed equivalent or more % release of drug as compared to the reference product. The stability study result of formulation F8 showed that the blister was the suitable package in comparison to the cold form blister packing. From the above studies it was found that the composition of formulation F8 can be recommended for further pharmacokinetic and pharmacodynamic studies in suitable animal models.

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