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Formulation development, evaluation and accelerated stability studies of entecavir tablet dosage form

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Abstract
In present investigation an attempt has been made to design and develop compressed tablet dosage form of entecavir drug. Optimization studies were done for the selection of glidant, lubricant and coating materials. Evaluation of granules was done on the basis of preformulation studies. Pre compression and post compression parameters were evaluated for optimization. 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 entecavir were analysed by high performance liquid chromatography (HPLC). Comparative dissolution studies and assays between optimized formulation and reference product showed better release and equivalent drug content. 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. The drug used in this formulation is used for the treatment of Hepatitis-B. Lactose monohydrate is used as diluent in tablet formulation which is safe to diabetic patients.

Keywords: Entecavir, Hepatitis-B, Direct compression, Blister packing, Lactose monohydrate.

1. Introduction
The vast majority of all tablets manufactured are made by compression, and compressed tablets are the most widely used dosage form in world [1]. Compressed tablets are prepared by the application of high pressures, utilizing steel punches and dies, to powders or granulations. Tablets can be produced in a wide variety of sizes, shapes and surface markings, depending upon the design of the punches and dies [2, 3].
The compressed tablet is the most popular dosage form in use today. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets [4]. A tablet can be formulated to deliver an accurate dosage to a specific site; it is usually taken orally [5]. The tablet is just one of the many forms that an oral drug can take such as syrups, elixirs, suspensions, and emulsions. Medicinal tablets were originally made in the shape of a disk of whatever color their components determined, but are now made in many shapes and colors to help distinguish different medicines [6, 7].
An Abbreviated New Drug Application (ANDA) contains data which when submitted to FDA's Centre for Drug Evaluation and Research, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product. Once approved, an applicant may manufacture and market the generic drug product to provide a safe, effective, low cost alternative to the American public [8].
Hepatitis is the Greek term for liver inflammation caused by certain viruses and other factors, such as alcohol abuse, some medications and trauma and also refers to a group of viral infections that affect the liver [9]. Viruses that can cause injury to liver cells include the Hepatitis A and Hepatitis C viruses. These viruses are not related to each other or to hepatitis B virus and differ in their structure, the ways they are spread among individuals, the severity of symptoms they can cause, the way they are treated, and the outcome of the infection. There are four major types of viral hepatitis, all caused by different viruses: Hepatitis A, Hepatitis B, Hepatitis C and Delta Hepatitis. This Study focuses on Hepatitis B. Viral hepatitis is the leading cause of liver cancer and the most common reason for liver transplantation. Although many cases of hepatitis are not a serious threat to health, infection with certain hepatitis viruses can become chronic (long-lasting) and can sometimes lead to liver failure and death. An estimated 4.4 million Americans are living with chronic hepatitis; most do not know they are infected [10-14].

The aim of the present work is to formulate, optimize, evaluate and dissolution method development & validation of tablet dosage form of entecavir with marketed brand.

2. Material and methods

After preformulation study it was found that dry granulation method for tablet manufacture is selected for the Entecavir formulation. The optimized amount of ingredients required for the preparation of each tablet is given in table 1. Accurately weighed ingredients were sifted through 40 mesh sieve. All the ingredients except magnesium stearate were blended for 30 min using an octagonal blender at slow speed (Kalweka VDM, Gujrat, India). Mill the granules through multimill with 1.0/1.5 mm screen and size the milled material through 30 mesh sieve. All the granules should pass through 30mesh. Load the sized granules in blender along with presifted lactose monohydrate and blend for 20 minutes. Lubricate the above blend with pre-sifted magnesium stearate in blender for 5 minutes. The granules were compressed into tablets using compression machine (Cadmach Machinery Co. Pvt. Ltd., Ahmedabad, India). Temperature below 25 ºC and relative humidity between 45-55% RH was maintained throughout the manufacturing process [15].

Table 1: Optimized formula for the preparation of entecavir tablets.

Ingredients*	Quantity (mg/tablet)
Entecavir	1.0
Microcrystalline Cellulose USP	62.0
Crospovidone USP	6.0
Povidone USP	10.0
Lactose Monohydrate USP	120.0
Magnesium Stearate USP	1.0
Total weight	200
Film Coating	
Opadry 04F58804 white INH	5.0
Purified Water IP	q.s
Each Tablet contain color: Titanium dioxide	

2.1 Selection of Diluent, Glidant and Lubricant

Diluent are used to increase the bulk of the formulation. Different diluents were tried for the optimization in the formula of formulation which included in table 2. 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 entecavir tablets for the selection of suitable one [16, 17].

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 in 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 [18].

Table 2: Manufacturing Formula For suitable Diluents Selection

Ingredients*	F1	F2	F3	F4	F5	F6	F7	F8
Entecavir	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sorbitol	120	--	--	--	--	--	--	--
Sorbitol P 60 W	--	120	--	--	--	--	--	--
Mannitol USP	--	--	120	--	--	--	--	--
Sucrose	--	--	--	120	--	--	--	--
Lactose Monohydrate USP	--	--	--	--	120	--	--	--
Lactose Monohydrate USP T/60	--	--	--	--	--	120	--	--
Lactose Monohydrate USP T/70	--	--	--	--	--	--	120	--
Lactose Monohydrate USP T/80	--	--	--	--	--	--	--	120
Other Excipients								
Microcrystalline Cellulose USP	62.0	62.0	62.0	62.0	62.0	62.0	62.0	62.0
Crospovidone USP	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Povidone USP	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Magnesium Stearate USP	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total weight	200	200	200	200	200	200	200	200

*all ingredients are in mg.

Table 3: Manufacturing Formula For lubricant Selection.

Ingredients*	F9	F10	F11
Entecavir	1.0	1.0	1.0
Lactose Monohydrate USP T/80	120	120	120
Crospovidone USP	6.0	6.0	6.0
Povidone USP	10.0	10.0	10.0
Microcrystalline Cellulose USP	62.0	62.0	62.0
Extra granular			
Magnesium Stearate USP	1.0	--	--
Zinc Stearate USP	--	1.0	--
Stearic acid USP	--	--	1.0
Total weight	200	200	200

2.2 Evaluation of granules

2.2.1 Bulk Density

The granules were passed through #40BSS 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,

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

2.2.2 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

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

2.2.3 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:

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

2.2.4 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:

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

2.2.5 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 [19]. 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.

2.2.6 Evaluation of dosage form

The manufactured tablets were evaluated for description, thickness, hardness, friability, weight variation, and disintegration time and dissolution test [20, 21].

2.2.7 Dissolution study

Dissolution study was carried out in official compendia by using USP II (Paddle type) dissolution apparatus [22]. The dissolution media containing potassium phosphate buffer (pH 6.8) was taken in each vessel of the apparatus. The volume of compendia were used 1000 mL and the temperature of the media was regulated at 37 °C ± 0.5 °C. Immediately one tablet was transferred into each vessel of the apparatus. The paddle was rotated at a speed of 50 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 45 min and the sampling was done at 10, 15, 30 and 45 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 entecavir [23, 24].

2.2.8 Accelerated Stability study

Optimized batch F8 was packed in blister pack (ALU –ALU), Cold Form Blister and was placed for stability study at 25 ˚C±2 ˚C/65%RH±5% RH and 40 ˚C±2 ˚C /75% RH±5%RH for 2 months. Sample was collected at every 1 month interval and evaluated for description, water content and assay stability study to show the effect of storage on these parameters [25]. (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 [26].

2.2.9 HPLC method of analysis

The released entecavir 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 degassed distilled water/methanol (80:20) with a flow rate of 1.0 ml/min. The injection volume was 100 µl, run time was 8 mint and elute was analysed at 253 nm.

3. Results and discussion

After preformulation studies it was found that dry granulation method was selected for the entecavir 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.

3.1 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) were performed. These granules were compressed into tablets and further evaluated for the parameters including weight, hardness, thickness, friability, and disintegration time.

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.

3.2 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 4. These granules were further compressed into tablets and evaluated for weight (mg), hardness (KP), thickness (mm), friability (%), and disintegration time (min.) respectively.

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 entecavir tablets.

Table 4: The results of Blend Parameters of selection of diluents.

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner's Ratio	Angle of repose (degrees)
F1	0.464	0.539	14.46	1.16	27.0
F2	0.469	0.561	16.39	1.19	36.6
F3	0.478	0.586	18.43	1.22	31.3
F4	0.480	0.637	24.61	1.32	35.6
F5	0.446	0.560	20.35	1.35	36.5
F6	0.462	0.591	21.80	1.27	36.6
F7	0.451	0..565	20.17	1.25	38.1
F8	0.475	0.565	16.03	1.19	31.6

3.3 Dissolution studies

The dissolution studies of entecavir tablets were performed and evaluated. The cumulative amount of drug released (%) at different time intervals was estimated for entecavir tablets of different composition and the innovator tablet which is shown in Figure 1. The composition of entecavir tablets (F1 to F8) was same and magnesium stearate was included as lubricant with each formula of tablets.

Table 5: Comparative dissolution test result of all Formulations and reference drug

S. No.	Time (min.)	%CDR								
		F1	F2	F3	F4	F5	F6	F7	F8	R*
1	10	92.6	90.2	78.9	86.5	85.4	87.6	83.1	94.2	95.6
2	15	95.2	93.3	89.4	91.3	89.3	90.3	86.6	96.1	97.7
3	30	96.4	94.8	93.2	95.8	96.7	94.2	92.2	98.6	97.9
4	45	97.9	97.9	95.3	96.9	98.4	97.8	98.5	100.2	98.2

All the tablets were found to release more than 90% after 30 min. Maximum release was found to be 98.6 % by F8 formulation. However, the reference tablet was found to release a maximum of 97.9% of drug. Thus, the release (%) of formulation F8 was better than the reference drug.

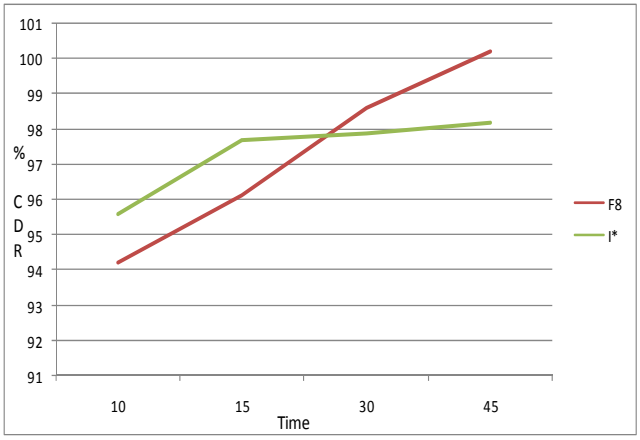

Fig 1: Comparison of In-vitro Dissolution Profile of Formulation (F8) and Reference

3.4 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 6. It was found that the entecavir tablets are more stable in the ALU-ALU blister pack at the both of the temperature and relative humidity of stability period.

Table 6: Stability Studies of Formulation F8

Cold Form Blister (25°C± 2°C/65% RH ± 5% RH)			
Stability study evaluation data. Months	**Description**	**Water content (%)w/w by KF**	**Assay Entecavir (mean)**
1	Red	5.66	102
2	Red	4.26	99.80
Cold Form Blister (40°C ± 2°C/75% RH ± 5% RH)			
1	Red	5.66	102
2	Red	4.30	99.25
ALU-ALU Blister (25°C ± 2°C/65% RH ± 5% RH)			
1	Red	5.66	102
2	Red	5.60	101.8
ALU-ALU Blister (40°C ± 2°C/75% RH ± 5% RH)			
1	Red	5.66	102
2	Red	5.56	101.2

4. Conclusion

The present investigation was carried out to develop a tablet dosage form of entecavir. Melt granulation method was found to be most suitable method for formulation of low dose dosage form in comparison of wet granulation. Lactose monohydrate was found to be suitable diluent for the present tablet dosage form and shows 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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