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Research Article

STUDIES IN PROSPECTIVE PROCESS VALIDATION OF MULTI COMPONENT ANTI-RETRO VIRAL TABLET DOSAGE FORMULATION

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ABSTRACT

Check the weight of all ingredient**.** Lamivudine, Zidovudineand PVPK-90were sifted through quadro co mill, sifted materials were collected in a double lined polybag and labelled as SIFT-A. Sodium starch gllycolate and Eudragit were sifted through quadro co-mill, sifted material were collected in a double lined polybag and labelled as SIFT-B. Dispensed Pectin was sifted through quadro mill. Sifted material was collected in double lined polybag and labelled as SIFT-C. Blending of SIFT-A and SIFT-B was done for 30 minutes at 14 rpm in 300L blender. After this, lubricant (SIFT-C) was added to the above blended material and blended again for 5 mins. After this sampling was done, 3 sets of 10 samples were taken from ten different locations of the blender for blend uniformity analysis and 150g of the sample was taken for bulk density, tap density and particle size distribution analysis.To calculate the Compressibility index and Hausner ratio. Compression was done by 26 stations compression machine, using tooling: Sifted material i.eLamivudine + Zidovudine + PVPK-90 and Sodium starch gllycolate + Eudragitwere loaded into V-blender and blended for 30 min at 14 rpm. Sifted magnesium stearate was then added to V-blender and was mixed for 5 min at 14 rpm. 3 sets of 6 samples were withdrawn from six different location of the v-blender to check the bulk uniformity along sample up to 150g Sampling was done after 30+5min of lubrication. The final blended material was loaded into double lined polybags and was marked as ready for compression. Accurately weighed and transferred about 70mg of Lamivudine working standard and 140mg of Zidovudine working standard into 50ml volumetric flask, add about 10ml of methanol and Sonicate to dissolve, make up the volume with dissolution medium and mix, further dilute 3ml to 25ml with dissolution medium and mix, flteredthrough 0.45*u*m nylon filter.The raw dissolution data was calculated for calculating the amount of drug released and percentage cumulative drug released at different time intervals.

KEY WORDS: Tablet, Lamivudine, Eudragit,starch gllycolate,Zidovudine,PVPK-90.

1. INTRODUCTION

The trial batch was followed after getting all information from Research and Development. It involved transfer of knowledge and transfer of technology. From blending to film coating each process had its set of challenges. The development of process through use of DOE (design of experiment) as well as understanding the critical vs non-critical parameters for each operation, are determining factors for success and failure of trial batch.

Table 1: Formula for trial batch tablet formulation

Ingredients	Quantity (kg)
Lamivudine	22.500
Zidovudine	45.000
PVPK-90	38.812
Sodium starch gllycolate	0.563
Eudragit	4.500
Pectin	1.125
HPMC	2.925
Purified water	26.33

Stage 1 Dispensing

- Check the weight of all ingredients.

Stage 2Sifting

- All dispensed material were verified
- Lamivudine, ZidovudineandPVPK-90were sifted through quadro co mill, sifted materials were collected in a double lined polybag and labelled as SIFT-A.
- Sodium starch gllycolate and Eudragit were sifted through quadro co-mill, sifted material were collected in a double lined polybag and labelled as SIFT-B.
- Dispensed Pectin was sifted through quadro mill. Sifted material was collected in double lined polybag and labelled as SIFT-C.

Stage 3 Blending

- Blending of SIFT-Aand SIFT-B was done for 30 minutes at 14 rpm in 300L blender.
- After this, lubricant (SIFT-C) was added to the above blended material and blended again for 5 mins.

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➢ After this sampling was done, 3 sets of 10 samples were taken from ten different locations of the blender for blend uniformity analysis and 150g of the sample was taken for bulk density, tap density and particle size distribution analysis.To calculate the Compressibility index and Hausner ratio.

Table 2: Parameters governing flow property

Compressibility index	Flow character	Hausner ratio
≤10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very poor	1.46 – 1.59
>38	Very, very poor	>1.60

➢ Occupancy of the blender was found by calculating the bulk density of the blend, occupancy should be 40-60% ± 10%, for the proper blending of the material.

Stage 4 Compression

➢ Compression was done by 26 stations compression machine, using tooling:

Upper punch:-capsule shaped and embossed with 'RX923'.

Lower punch:- capsule shaped and plain.

➢ Various parameters were observed at pre start, start, middle and end of the compression. And about 60 tablets were sent for analysis to check there dissolution rate (DR), disintegration time (DT) and content uniformity (CU).

Table 3: Parameters set for the tablet

Parameters	Value Range
Machine speed (rpm)	20 - 40
Average weight (mg)	735 – 765
Uniformity of weight (mg)	712.5 – 787.5
Thickness (mm)	5.2 - 5.8
Hardness (kp)	10 – 25
Friabilty(% w/w)	Not more than 1.0
Disintegration time (min) 37^0C water	Not more than 15 mins

Stage 5 Film Coating

➢ Coating suspension was made by mixing film coating agent and purified water in a stainless steel vessel with continous stirring. This stirring was continued for 45 minutes till a homogenous suspension was obtained.

➢ Before starting the coating process guns were validated to ensure that equal volume of coating solution is coming out from each gun and there is no hindrance in flow from three of the spray guns, for this solution is collected in sample polybags for fixed time from 3 guns and weighed.

➢ Tablets were loaded into the Ganson coater for pre warming.

Table 4: Pre-warming coating parameter

Parameters	Set
Inlet temperature (^{0}C)	60 – 70
Bed temperature (^{0}C)	Not less than – 45
Pan speed (rpm)	Jogging for 5 min. at 2 rpm

➢ After pre-warming average weight of 300 tablets was taken and recorded as 746.67mg.

➢ After achieving desired bed temperature, film coating was started and was continued until 2.25% of weight buildup was achieved.

➢ When weight buildup was achieved dosing was turned off and tablet bed was dried at 60^0C inlet temperature, for 5 minutes with pan speed of 2 rpm.

➢ After drying, inlet blower was turned off and tablet bed was set off for cooling to achieve product bed temperature below 27^0C with intermittent jogging of pan at 2 rpm.

➢ Tablets were unloaded into double lined polybags and finished product sample were sent for dissolution rate profile analysis.

Table 5: Parameters set for film coating

Parameters	Set
Number of guns	3
Gun to bed distance	22 - 26
Nozzle size (Dia) (mm)	1.0
Atomizing pressure (Kg/cm^2)	2.5 - 4.5
Pump (rpm)	10 - 25
Inlet temperature (^{o}C)	60 - 70
Outlet temperature (^{o}C)	40 - 60
Bed temperature (^{o}C)	40 - 60
Pan speed (rpm)	2 – 7
Spray rate (g/min)	80 -300

2. VALIDATION BATCH PREPARATION:

The trial batch results revealed that the recommended manufacturing produces the product complying with designed specification. Based on the result of the trial batch study it was concluded that the quality of subject product can be predicted with a high degree of assurance to meet the set designed standard when manufactured using the proposed manufacturing process.Thus the results were found good and the process was proposed for validation. Therefore protocol for the validation was designed. Three consecutive validation batch will be executed. The batch sizeof validation batch will be same to the batch size of comercial production batch,equipments validated will only be used for commercial batch of the product.

Table 6: Formula for validation batch tablet formulation

Ingredients	Quantity (kg)
Lamivudine	80.000
Zidovudine	160.000
PVPK-90	135.250
Sodium starch gllycolate	2.250
Eudragit	18.00
Pectin	4.500
HPMC	11.400
Purified water	105.000

3. Evaluation

3.1. Pre-compression Parameters:

- Bulk density and Tapped density

Granule density may infleunce compressibility, tablet porosity, dissolution and other properties. The term bulk density and tapped density refers to a measure used to describe a packaging of particles or granules. The equation for determining bulk density($\boldsymbol{\rho_b}$)= M/ V_b, where M is the mass of the particles and V_b is the total volume of packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device that has a specially cut rotating cam, in this initial volume is noted and the sample is tapped until no further reduction in volume is noted, a sufficient number of taps should be employed to assure reproducibility for the material mainly 1250 number of taps are performed. The equation

for determining tapped density (ρ_t) = M/V_t, where V_t is the tapped volume of packing.

- Compressibility index and Hausner's ratio

An important measures that can be obtained from the bulk density determination is thecompressibility Index and HausnerRatio,which are defined as

Compressibility Index (C) = $\rho_t - \rho_u/\rho_t X100$, where $\rho_u$is the untapped bulk density (often called Loose or Aerated bulk density).

Hausner Ratio = ρ_t / ρ_u, where $\rho_t$is the tapped density (often called tight density) and$\rho_u$is the untapped bulk density (often called Loose or Aerated bulk density).

According to theory, the more compressible a bed of particulates is, the less flowable the powder or granulation will be,conversly the less compressible a material is the more flowable it will be. Bulk density largely depends on particle shape, as the particles became more spherical in shape, bulk density is increased. In addition, as granule size increases, bulk density decreases, the smaller granules are able to form a close, more intimate packing than larger granules.

- **Particle size distribution**

The particle size of a granulation is known to affect the average tablet weight, tablet weight variation and disintegration time.

To determine the particle size, about 100g of blend is weighd and passed through seive number #22, #36, #60, #100 and powder retained on each sieve and pan is calculated. The equation used for determining particle size distribution is as follows:

Particle size distribution (PSD) = **Mass retained on the sieve / total mass obtained X 100**

- **Blend uniformity**

Sifted material i.eLamivudine + Zidovudine + PVPK-90 and Sodium starch gllycolate + Eudragitwere loaded into V-blender and blended for 30 min at 14 rpm. Sifted magnesium stearate was then added to V-blender and was mixed for 5 min at 14 rpm. 3 sets of 6 samples were withdrawn from six different location of the v-blender to check the bulk uniformity along sample up to 150g Sampling was done after 30+5min of lubrication. The final blended material was loaded into double lined polybags and was marked as ready for compression.

3.2 Procedure for assay and blend uniformity analysis:

Preparation of phosphate buffer (pH 7.0):- Transferred 5.43g of potassium dihydrogen orthophosphate in 4000ml of water. Added 4ml of triethylamine and adjusted the pH 7.0 with orthophosphoric acid, and filtered through 0.45*u*m membrane filter.

Preparation of mobile phase: Mixed 3500ml of buffer pH 7.0 and 1500ml of methanol.

Preparation of diluent: Mixed 7500ml of water and 2500ml of methanol.

3.3 Preparation of standard solution: Approx. 60mg of API 1 and 120mg of API 2 was transferred into 100 ml volumetric flask, add about 50ml of diluent. Sonicate for about 10 min with intermittent shaking. Making up the volume with diluent, further dilute 10ml to 100ml with diluent, filter the solution 0.45um membrane filter and was put into the vial. First 2-3ml solution passing from membrane filter was discarded.

3.4 Preparation of sample solution for blend uniformity analysis: Quantitatively transfer the contents of blend to 1000ml volumetric flask, add about 500ml of diluent. Sonicate for about 10 min with intermittent shaking. Making up the volume with diluent and mixed properly. Further dilute 10ml to 100ml with diluent, filter the solution through 0.45um nylon filter and was put into the vial. First 2-3ml solution passing from nylon filter was discarded. Repeat the operation for all the other 6 samples collected for blend uniformity analysis.

3.5 Preparation of sample solution for assay analysis: Quantitatively transfer about 750mg of blend to 250ml volumetric flask, add about 125ml of diluent. Sonicate for about 10 min with intermittent shaking. Making up the volume with diluent and mixed properly. Further dilute 10ml to100ml with diluent, filter the solution through 0.45um nylon filter and was put into the vial. First 2-3ml solution passing from nylon filter was discarded.

3.6 Chromatographic parameters: Waters HPLC with EMpower software

Table 7: Parameters set for HPLC (for blend uniformity)

Column	Kromesil C-18
Column oven temperature	30^{o}C
Flow rate	1.0ml/min
Detector	UV at 266nm
Injection volume	20*uL*
Run time	10 min

4. PROCEDURE FOR CONTENT UNIFORMITY ANALYSIS:

Preparation of phosphate buffer (pH 7.0):- Transferred 5.43g of potassium dihydrogen orthophosphate in 4000ml of water. Added 4ml of triethylamine and adjusted the pH 7.0 with orthophosphoric acid, and filtered through 0.45um membrane filter.

Preparation of mobile phase: Mixed 3500ml of buffer pH 7.0 and 1500ml of methanol.

Preparation of diluent: Mixed 7500ml of water and 2500ml of methanol.

4.1 Preparation of standard solution: Approx.60mg of Lamivudine and 120mg of Zidovudinewas transferred into 100 ml volumetric flask, add about 50ml of PVPK-90. Sonicate for about 10 min with intermittent shaking. Making up the volume with PVPK-90, further dilute 10ml to 100ml with PVPK-90, filter the solution 0.45um membrane filter and was put into the vial. First 2-3ml solution passing from membrane filter was discarded.

4.2 Preparation of sample solution: Transfer one intact tablet to a 250ml volumetric flask, add 125ml of PVPK-90Sonicate for about 10min and make up volume with PVPK-90 further dilute 10ml to 100ml with PVPK-90, filter through 0.45*u*m or finer porosity membrane filter. Repeat the operation on nine other tablets.

4.3 Chromatographic parameters: Waters HPLC with EMpower software

Table 8: Parameters set for HPLC (for content uniformity)

Column	Kromesil C-18
Column oven temperature	30^{o}C
Flow rate	1.0ml/min
Detector	UV at 266nm
Injection volume	20*uL*
Run time	min

4.4 *In-vitro* dissolution test:

Dissolution study of tablet performed in USP II (paddle) dissolution apparatus using 900ml of 0.1N HClas a dissolution media. The tablet was loaded into each basket of dissolution apparatus; the temperature of dissolution media was maintained at 37^{0}C ± 0.5^{0}C with stirring speed of 75 rpm throughout the study. Aliquots of dissolution media containing 10ml of samples were withdrawn at time interval of 10, 15, 20, 30, 45 minutes and 5ml of fresh dissolution media maintained at the same temperature was replaced after each withdrawal. The samples were analyzed by HPLC method. The raw dissolution data was calculated for calculating the amount of drug released and percentage cumulative drug released at different time intervals.

5. EVALUATION OF THE FORMULATION PARAMETERS:

5.1 Trial batch

Table 9:- Resultof bulk density, tapped density, particle size distribution and blend uniformity of trial batch.

Bulk density (g/ml)	0.56
Tapped density (g/ml)	0.75
Carr's Index	25.3
Hausner Ratio	1.33
Particle size distribution (100.14g)	
Mesh size	% Retained
# 22	0.29
# 36	1.12
# 60	5.62
# 100	16.83
Pan	76.08

Bulk uniformity (% of claim)		
Sampling locations	API 1	API 2
1	99	102
2	97	100
3	98	102
4	98	102
5	95	103
6	100	101
Average	98	102

Blending was done in a non-shear blender(v-blender) with occupancy of approx 70% for 30 min at 14rpm followed by lubrication for 5min at 14rpm. Blend uniformity analysis individual result was found in the range of 97% to 100% forLamivudine and 100% to 102% for Zidovudine.

Table 10:- Result of content uniformity of compressed tablets of trial batch

Content uniformity (% of claim)						
Sample	Start of compression		Middle of compression		End of compression	
	Lamivudine	Zidovudine	Lamivudine	Zidovudine	Lamivudine	Zidovudine
1.	96.7	102.8	98.4	102.4	101.4	102.5
2.	97.5	102.0	104.2	100.2	101.4	101.8
3.	98.0	100.9	100.7	101.3	99.2	102.0
4.	99.0	102.1	102.2	101.7	100.3	102.5
5.	99.2	102.2	102.9	101.5	98.5	101.5
6.	98.6	101.5	99.8	103.0	101.9	105.0
7.	101.0	102.4	101.7	101.9	98.7	103.6
8.	98.9	102.4	102.3	101.3	99.9	103.4
9.	99.9	102.9	103.4	102.4	99.6	103.3
10.	97.7	102.8	103.2	100.6	100.6	101.8
Average	*98.6*	*102.1*	*101.9*	*101.6*	*100.1*	*102.7*
SD	*1.30*	*0.77*	*1.79*	*0.85*	*1.25*	*1.09*
AV[*]	*3.1*	*2.3*	*5.0*	*2.0*	*4.7*	*3.9*

[*] ***Acceptance value should not be more than 15.***

Content uniformity during start, middle and end of compression run was found to be 96.7% to 104.6% for Lamivudine and 100.2% to 101.5% for Zidovudine and based on the above observations it was confirmed that the values of uniformity of dosage units at different stages were within the acceptance criteria.

Table 11:- Dissolution Rate profile and Assay of 1st validation batch

Dissolution rate profile (% Drug Released) (Apparatus II / 0.1 N HCl / 75rpm / 900ml)										
Vessel	Time (in minutes)									
	Lamivudine					Zidovudine				
Time (min)	10	15	20	30	45	10	15	20	30	45
Vessel 1	99	102	102	102	100	99	101	102	102	103
Vessel 2	100	101	101	101	100	99	100	101	102	102
Vessel 3	99	100	102	102	102	100	102	103	103	103
Vessel 4	99	102	100	101	99	98	100	101	101	100
Vessel 5	101	101	102	101	101	100	102	102	103	102
Vessel 6	101	102	101	102	101	101	100	102	103	102
F2 value	59	63	67	52	57	59	68	62	56	52
Assay	100.4 %					100.1 %				

Average release of Lamivudine and Zidovudinewas found upto 100% drug release within 10 min. The finished product analytical result conclude that the observed coated tablet parameters were within range as per product release specification.

Table 12:- Dissolution Rate profile and Assay of 3rd validation batch

Dissolution rate profile (% Drug Released) (Apparatus II / 0.1 N HCl / 75rpm / 900ml)										
Vessels	**Time (in minutes)**									
	Lamivudine					**Zidovudine**				
Time (min)	**10**	**15**	**20**	**30**	**45**	**10**	**15**	**20**	**30**	**45**
Vessel 1	99	100	101	102	103	101	101	102	102	102
Vessel 2	101	102	102	103	103	99	100	103	103	103

Vessel 3	99	100	100	102	102	101	102	102	102	103
Vessel 4	99	99	100	101	101	99	100	102	102	102
Vessel 5	100	102	102	103	102	100	101	101	103	101
Vessel 6	99	101	101	101	101	99	101	101	102	102
F2 value	**52**	**59**	**62**	**65**	**74**	**51**	**60**	**53**	**71**	**51**
Assay	**100.3 %**					**100.1 %**				

Average release of Lamivudine and Zidovudinewas found more than 100% drug release within 10 min. The finished product analytical results conclude that the observed coated tablet parameters were within range as per product release specification.

6. CONCLUSION:

All the in-process and analytical results were found satisfactory and well within the specified acceptance criteria. The overall review of results shows consistency and reproducibility within and in between the batches. These results demonstrate that the manufacturing process was under the state of control throught all the stages within and in between batches and thus the process was validated and was ready for the commercial production of the batches.

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