

FORMULATION AND EVALUATION OF FIXED-DOSE COMBINATION OF BILAYER TABLETS OF ATAZANAVIR SULFATE AND RITONAVIR 300 MG/100 MG

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ABSTRACT

Objective: The objective of this study is to formulation and development of fixed-dose combination as a single dosage regimen by using the design of experiments (DOE) approach against the single dose of reference listed drugs of brand reyataz capsule 300 mg (atazanavir sulfate) and norvir tablets 100 mg (ritonavir tablets) to treat human immunodeficiency virus (HIV) Infections.

Methods: Formulation was developed with each blend of ritonavir by using hot-melt extrusion and atazanavir sulfate by wet granulation process and compressed by bilayer technology followed by film coating. Formulation and process optimization by design of experiments (DOE) to evaluate dissolution and related substances of the finished product. Fractional factorial (2^{2+3}) and full factorial design (3^{3+3}) by using a design expert (version 11.0) were used to evaluate the formulation and process variables to prepare a robust formulation.

Results: Results indicate that the sorbitan monolaurate range has played a key role to achieve the dissolution for ritonavir formulation. The studied temperature range and interaction of temperature and feed rate, temperature and screw speed during the hot-melt extrusion process impact on the related substances of the bi-layer tablet. Analysis of variance (ANOVA) also finding the P-value less than 0.0500 and the studied range was significant. Design space was established for the significant factors to control the results within the acceptable limits. The studied formulation and wet granulation process for atazanavir sulfate have no significant impact on dissolution and related substances of the finished product. Further, the studied hardness range of 16-28kp for bi-layer tablets has no critical impact on the dissolution. Optimum formulation and process of bi-layer tablets in F37 yielded similar drug release and related substances against the reference drug product.

Conclusion: The present invention of fixed-dose combination can be recommended as a single dosage regimen with the consistent drug release and control of the unknown impurities in the prototype formulation against the individual reference drug product.

Keywords: Fixed-dose combination formulation, Human immunodeficiency virus (HIV) infections, Bilayer technology, Quality by design (QbD), Design of experiments (DOE), 2^{3+3} and 2^{2+3} full factorial and fractional factorial design

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INTRODUCTION

Fixed-dose combination drugs can be defined as two or more drugs in a single formulation, each drug having independent modes of action, or the combination of which are synergistic or additive, or complementary in their effect. "Free" combinations can be defined as two or more drugs in separate formulations, each taken usually at the same time.

Fixed-dose combination (FDC) products are common in the treatment of hypertension, diabetes, human immunodeficiency virus, and tuberculosis. They make it possible to combine two or more drug molecules with different modes of pharmacological actions in a single dosing unit and optimize the treatment. From a patient perspective, they offer convenience, reduced dosing unit burden, and cost savings. From a clinical perspective, an aging population in developed countries will need multiple medications to treat age-related diseases and co-morbidities. Fixed-dose combination (FDC) products simplify dosing regimens and enhance patient compliance. The number of fixed-dose combination (FDC) products has grown over the years and the trend is likely to continue. While some formulation technologies such as multi-layer tablets, multiparticulate systems, active film coating, and hot-melt granulation. Historically, fixed-dose combination (FDC) products were developed for improved compliance, better efficacy, and reduced adverse events [1, 2]. In the current scenario, the strategies for developing a fixed-dose combination (FDC) are primarily based on the therapeutic requirements.

Assessments of the desired benefits such as patient adherence, enhanced efficacy, and better safety profiles compared to the existent drug therapies and possible limitations (e. g., cumulative toxicity) of the combination product are performed. Fixed-dose

combination (FDC) product development is governed by regulatory recommendations, where clinical, biopharmaceutical and pharmacokinetic (PK) considerations are critical for their development. One of the main biopharmaceutical considerations is the dose of each active substance in the fixed-dose combination (FDC), which should be appropriately evaluated and clinically justified.

Fixed-dose combinations (FDC) are generally developed with components having complementary mechanisms of action with a clear medical rationale. Typically this is the case for an uncontrolled disease or comorbid condition requiring multi-drug therapy resulting in pill burden. Following are the general clinical considerations for the development of fixed-dose combination (FDC) products [3].

A fixed-dose combination of drugs is considered to be essentially similar or bioequivalent to an innovator drug product. Due to the high costs of the innovator drug products, these are not affordable to the common people. So, the development of generic drugs is very important to afford to common people as well as to reduce treatment costs.

Generic companies are not needed to conduct non-clinical studies and phase I, phase II, and phase III clinical studies to explicit safety and efficacy of the drug products. Instead, generic companies need to show that their generic product is bioequivalent to that of the innovator drug product through bioequivalence studies, thereby making medicines more affordable and more accessible to wider populations preventing drug shortages. Development of fixed-dose formulations becomes very difficult, as the innovator companies used to protect the drug release dosage forms technology, composition, polymers, dissolution profile and biodata through

patents. The main challenge is developed fixed-dose formulation should pass bio-equivalency studies in fasting and fed conditions and also, the product should be stable and should meet international conference on harmonization (ICH) guidelines. The bio-availability and bio-equivalency will depend on the type of surfactants, solubilizer, diluents, type of polymer used, percentage of disintegrant, and technology used for manufacturing [4].

The quality by design (QbD) approach with the design of experiments (DOE) by statistical evaluation for the formulation and process variables is must prove the consistent results of the finished product. The objective of the design of experiments (DOE) by using full factorial and fractional factorial design is to evaluate the critical material and critical process attributes to affect the responses of the finished product and finally establish the design space for controlling the drug release and unknown impurities of prototype formulations. The current study was used the bilayer tablet concept for the compression of two layers such as ritonavir (Layer-I) and atazanavir sulfate (Layer-II) as a fixed-dose formulation. In addition, compatibility results also recommended to use of bilayer compression to control the related substances within the international conference on harmonization (ICH) guidelines [5-7].

MATERIALS AND METHODS

Pre formulation studies

The excipients used in atazanavir sulfate and ritonavir tablets were selected based on the excipients used in the reference drug products. A summary of the excipients-drug substance compatibility was assessed through high-performance liquid chromatography (HPLC) analysis of binary mixtures of excipients and drug substances at a 1:1 ratio in the solid state. Samples were stored at 25 °C/60% RH in closed conditions and 40 °C/75% RH in both open and closed containers for one 1month [8].

Based on the evaluation of drug and excipients compatibility study, there is the interaction between drug substances of ritonavir and atazanavir resulted in increases the unknown impurity and it was

not compatible. Hence, bilayer compression was recommended. Initial development for atazanavir formulation, direct compression was recommended and it was yielded the variation of assay and content uniformity issues and poor flow of the final blend was observed. Further, the wet granulation method was proposed to improve the content uniformity by controlling the flowability of the granules [9-11]. In the case of ritonavir formulation design, hot-melt extrusion is recommended based on the availability of literature data, previous experience and patents data [12-14].

Tooling details

Based on the weight and size of individual reference drug product such as reyataz capsule 300 mg (atazanavir sulfate) and norvir tablets 100 mg (ritonavir tablets), bilayer tablets of the test formulation was targeted to 1560.00 mg at compression stage by using punch tooling of 21.20X11.5 mm during the formulation and process development study.

Initial development of a fixed-dose combination of atazanavir sulfate and ritonavir bilayer tablets 300 mg/100 mg was planned with bilayer compression strategy considering ritonavir blend is Layer-I and atazanavir sulfate blend in Layer-II.

The rationale for selecting the manufacturing for atazanavir sulfate based on the fine particle size and poor flowability of active pharmaceutical ingredient (API), wet granulation process was confirmed. In the case of ritonavir, because of the solubility enhancement and patent research data, hot-melt extrusion was selected.

The initial proposal was to use similar qualitative composition as individual reference standards, but the final composition and manufacturing method was selected based on the physicochemical characteristics of the development product [15-17].

Based on the literature search and previous experience, the proposed manufacturing procedure for the formulation was designed as per fig. 1.

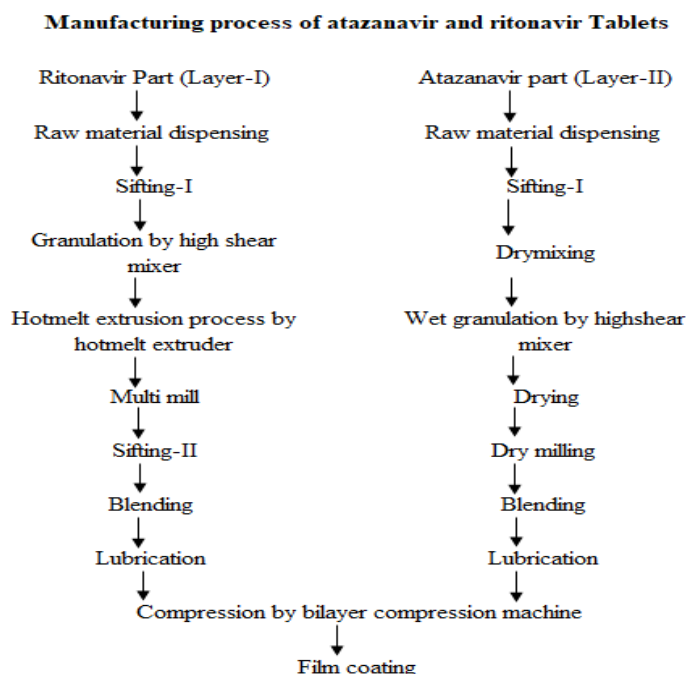


Fig. 1: Manufacturing process of fixed-dose combination (FDC) product

Dissolution method development

Based on the solubility of drug substances, it showed pH-dependent solubility across the physiological pH range. But maximum saturation solubility in acidic pH. So, dissolution media of 0.025 NHCL+0.06

MPOE10LE/900 ml/100 RPM was selected [18, 19]. Overall dissolution comparison within the formulation was carried out from the batches of F1 to F37. Hence, a hardness range from 16-26 N was maintained. Further, test VS reference product dissolution was compared to understand the differences in drug release between the formulations.

Experimental design

Formulation of ritonavir part (Layer-I)

Based on the available patents and reference product composition, ritonavir formulation was manufactured by using wet granulation of dry mix mixture followed by hot-melt extrusion process.

As per the composition table 3, the level of intragranular materials such as sorbitan monolaurate and colloidal silicon dioxide may affect the dissolution. Therefore, the level of intra granular will be determined through formulation optimization (design of experiments) DOE. Formulation of center point batches such as F12, F17, and F18 was observed to be the final composition. This final formulation was compared with other designs of experiment (DOE) batches as per table 3.

Sorbitan monolaurate was used as a solubilizer and the levels

investigated ranged from 40.00 mg to 80.00 mg. colloidal silicon dioxide is being used as anti-adherent in the formulation. It may promote the wicking action and ultimately affect dissolution; the levels investigated range from 0.00 mg to 16.00 mg.

These levels are considered based on the prior knowledge and inactive ingredient level (IID level) as per Food and drug administration (FDA) and are within the recommended range in the handbook of pharmaceutical Excipients [20].

During the evaluation of formulation design of experiments (DOE) design for the intragranular part of the ritonavir layer. The atazanavir layer of center point composition will be kept constant. Fractional factorial design with 2 factor, 2 level 2²+3 was selected for the formulation of ritonavir and atazanavir sulfate to evaluate the design of experiments by using a design expert software program [21, 22].

Table 1: Design of experiments (DOE) design for ritonavir target formulation (Layer-I)

Independent variables	Different levels		
	Low	Target	High
Sorbitan monolaurate (mg)	40.00	60.00	80.00
Colloidal silicon dioxide (mg)	0.00	8.00	16.00
Response	Goal	Acceptable Range	
Diss. in 0.025 N HCL+0.06 MPOE10LE	Maximize	NLT85 (Q)at 120 min.	

Formulation of atazanavir part (Layer-II)

In the case of layer-II, study the effect of two formulation factors such as starch 1500 and crospovidone on dissolution was identified as high risk for layer-II. During the evaluation of formulation design of experiments (DOE) design for the intragranular part of the

atazanavir layer. The ritonavir layer of center point composition will be kept constant.

Formulation of center point batches such as F23-F25 was observed to be the final composition. This final formulation was compared with other designs of experiment (DOE) batches as per table 4.

Table 2: Design of experiments (DOE) design for atazanavir target formulation (Layer-I)

Independent variables	Different levels		
	Low	Target	High
Starch 1500 (mg)	10.00	15.00	25.00
Crospovidone (mg)	10.00	15.00	25.00
Response	Goal	Acceptable Range	
Diss. in 0.025 N HCL+0.06 MPOE10LE	Maximize	NLT85 (Q)at 120 min.	

Starch 1500 was used as a diluent and the levels investigated ranged from 5.00 mg to 15.00 mg. Crospovidone was used as a disintegrant in the formulation. These two factors may affect dissolution; the levels investigated range from 5.00 mg to 15.00 mg.

While study for layer-I formulation optimization study as per table 3,

the final blend with the layer-II formulation of the center point batch (F23-F25) was used for the bilayer compression, compression was carried out by using both layers with a bi-layer compression machine (10 stations, Parle Elizabeth) for an average weight of 1560.00 mg. Further, all the design of experiments (DOE) batches was evaluated with a hardness range of 16-28 kP.

Table 3: Design of experiments (DOE) for ritonavir formulation (Fractional factorial design by a design expert, (2²+3))

S. No.	Ingredients	F12	F13	F14	F15	F16	F17	F18
		mg/tablet						
Intra granular materials								
1	Ritonavir	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2	Co povidone	585.00	573.00	557.00	597.00	613.00	585.00	585.00
3	Sorbitan monolaurate	60.00	80.00	80.00	40.00	40.00	60.00	60.00
4	Colloidal silicon dioxide	8.00	0.00	16.00	16.00	0.00	8.00	8.00
Extra granular materials								
5	Dicalcium phosphate anhydrous	110.00	110.00	110.00	110.00	110.00	110.00	110.00
6	Colloidal silicon dioxide	20.00	20.00	20.00	20.00	20.00	20.00	20.00
7	Sodium stearyl fumarate	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Total		890.00	890.00	890.00	890.00	890.00	890.00	890.00

Note: F012, F017, and F18 is target formulation of center point batches

While study for layer-II formulation optimization study as per table 4, the final blend with the layer-I formulation of the center point batch (F12, F17-F18) was used for the bilayer compression. Both blends of layer-I and II were used for the bilayer

compression machine (10 stations, Parle Elizabeth) for an average weight of 1560.00 mg. Further, all the design of experiments (DOE) batches were evaluated with a hardness range of 16-28kP.

Table 4: Design of experiments (DOE) of atazanavir formulation (Fractional factorial design by a design expert, (2²+3))

S. No.	Ingredients	F19 mg/tablet	F20	F21	F22	F23	F24	F25
Intra granular materials								
1	Atazanavir sulfate	341.70	341.70	341.70	341.70	341.70	341.70	341.70
2	Lactose monohydrate	162.00	147.00	147.00	132.00	152.00	152.00	152.00
3	Starch 1500	10.00	25.00	10.00	25.00	15.00	15.00	15.00
4	Crospovidone	10.00	10.00	25.00	25.00	15.00	15.00	15.00
Extra granular materials								
5	Starch 1500	50.00	50.00	50.00	50.00	50.00	50.00	50.00
6	Crospovidone	50.00	50.00	50.00	50.00	50.00	50.00	50.00
7	Calcium silicate	40.00	40.00	40.00	40.00	40.00	40.00	40.00
8	Ferric oxide yellow	0.50	0.50	0.50	0.50	0.50	0.50	0.50
9	Magnesium stearate	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Total		670.00	670.00	670.00	670.00	670.00	670.00	670.00

Note: F023, F024, and F25 are target formulations of center point batches.

RESULTS

Atazanavir sulfate and ritonavir are biopharmaceutical classification systems (BCS) class II and class IV compounds, respectively, with low solubility. Atazanavir sulfate is officially in the international pharmacopoeia; ritonavir is official in United States pharmacopoeia (USP) and international pharmacopoeia. The drug product, atazanavir,

and ritonavir tablets, are not officially in any of the pharmacopoeia. Hence, the dissolution method of 0.025 NHCL+0.06 M POE10LE was selected based on the solubility studies of both active substances and patent literature studies. Hence, the studied design of experiments (DOE) batches was performed in the dissolution media to understand the significant differences of formulation variables of both layers. Table 5 and table 6 indicates dissolution is the main response.

Table 5: Factors and response of design of experiments (DoE) design for the ritonavir intragranular formulation (Layer-I)

Batch No.	Standard	Run	Factor 1 A: Span 20 (mg)	Factor 2B: aerosil (mg)	Response 1: Dissolution of ritonavir at 120 min (%)*
F12	5	2	60	8	92±0.78
F13	2	1	80	0	98±0.68
F14	4	3	80	16	98±0.42
F15	3	4	40	16	82±1.20
F16	1	5	40	0	84±0.82
F17	6	6	60	8	95±0.34
F18	7	7	60	8	95±0.58

*Data given in mean±SD.

Table 6: Factors and response of design of experiments (DOE) for the atazanavir intragranular formulation (Layer-II)

Batch No.	Standard	Run	Factor 1A: Starch1500 (mg)	Factor 2B: crospovidone (mg)	Response 1: dissolution of atazanavir at 120 min (%)*
F19	3	1	5	5	96±0.26
F20	5	2	25	5	97±1.40
F21	1	3	5	25	97±0.68
F22	4	4	25	25	97±0.89
F23	6	5	15	15	96±1.60
F24	2	6	15	15	98±0.42
F25	7	7	15	15	97±1.20

*Data given in mean±SD

The dissolution method was tested using the in-house method. All the batches were meeting Q point of the dissolution (Q>80% dissolved in 120 min); since center points were included in the design of experiments (DOE), the significance of the curvature effect was tested using an adjusted model. Analysis of variance (ANOVA)

analysis represents whether a model and factor are significant for the respective response or not. It is concluded to be significant if the p-value were below the threshold (p = 0.05). The analysis of variance (ANOVA) results are presented below and it defines the formulation variables are significant or not on the final product.

Table 7: Analysis of variance (ANOVA) results of the model adjusted for curvature effect (Layer-I)

Response	Source	Sum of squares	df	Mean square	F value	P-value*	Comments
Diss. in	Model	227.00	3	75.67	25.22	0.0384	Significant
0.025 N	A-Sorbitan monolaurate	225.00	1	225.00	75.00	0.0131	Significant
HCL+	B-Colloidal silicon dioxide	1.00	1	1.00	0.3333	0.6220	Not significant
0.06 M	AB	1.00	1	1.00	0.3333	0.6220	Not significant
POE	Curvature	21.00	1	21.00	7.00	0.1181	Not significant
10LE	Pure error	6.00	2	3.00	-	-	-
	CorTotal	254.00	6	-	-	-	-

*P-value less than 0.0500 indicate above model terms and factor-A are significant

Table 8: Analysis of variance (ANOVA) results of the model adjusted for curvature effect (Layer-II)

Response	Source	Sum of squares	df	Mean square	F value	P-value*	Comments
Diss. in	Model	0.7500	3	0.2500	0.2500	0.8576	Significant
0.025 N	A-Starch1500	0.2500	1	0.2500	0.2500	0.6667	Not significant
HCL+	B-Crospovidone	0.2500	1	0.2500	0.2500	0.6667	Not significant
0.06 M	AB	0.2500	1	0.2500	0.2500	0.6667	Not significant
POE	Curvature	0.1071	1	0.1070	0.1070	0.7745	Not significant
10LE	Pure error	2.00	2	1.0000	-	-	-
	Cor Total	2.86	6	-	-	-	-

* P-value less than 0.0500 indicate above model terms are significant

Based on the analysis of variance (ANOVA) results for ritonavir (Layer-I), the model and sorbitan monolaurate showed a significant impact on the dissolution, and the P-value was observed less than 0.0500. Colloidal silicon dioxide has no impact on the dissolution and curvature and interaction of both factors is also observed to be not significant. The overlay plot indicates that the green zone is found to be within the specification limit and the grey zone indicates a failure to meet the specification. Further, the half-normal plot and contour plot also represents sorbitan monolaurate has a significant

impact on the dissolution. Hence, sorbitan monolaurate in the formulation is recommended to 47 to 80 mg from 40-80 mg/tablet for controlling the consistency of finished product dissolution results. Atazanavir sulfate (layer-II), model showed no significant impact on the dissolution, and P-value was also observed at more than 0.0500. It indicates that the studied range of both factors has no significant impact on the dissolution and all the results were found to be well within the limit. Hence the overlay plot showed all the green.

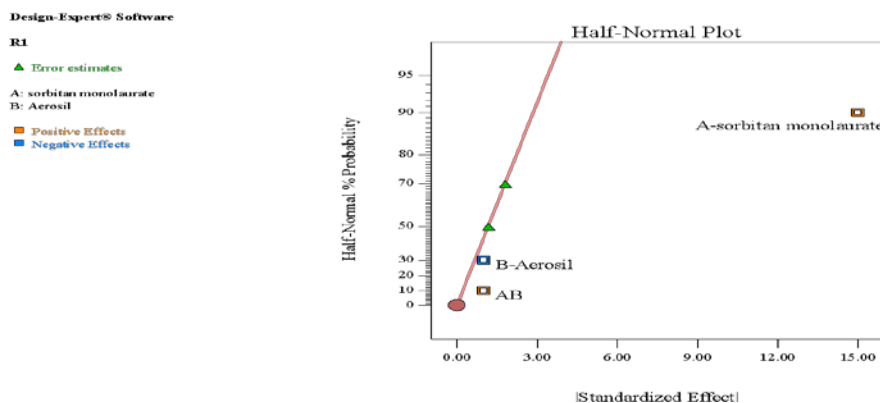


Fig. 2: The half-normal plot of the formulation variable effects on dissolution rate (Layer-I)

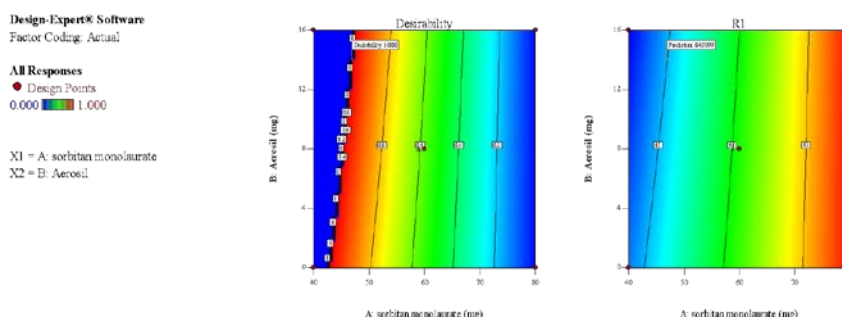


Fig. 3: Contour plot of the formulation variables on dissolution (Layer-I)

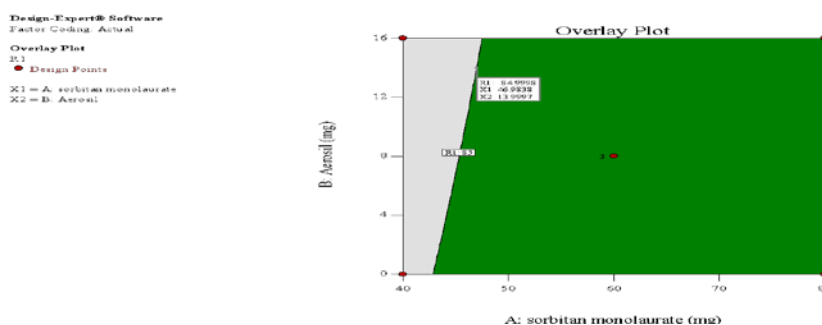


Fig. 4: Overlay plot-effect of formulation variables on responses (Layer-I)

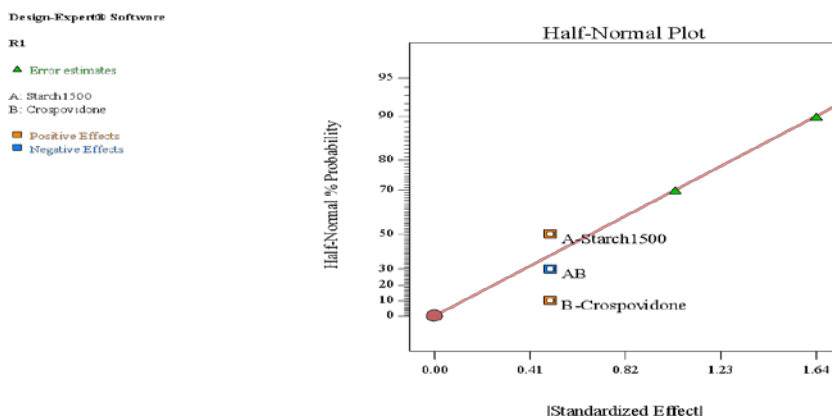


Fig. 5: The half-normal plot of the formulation variable effects on dissolution rate (Layer-II)

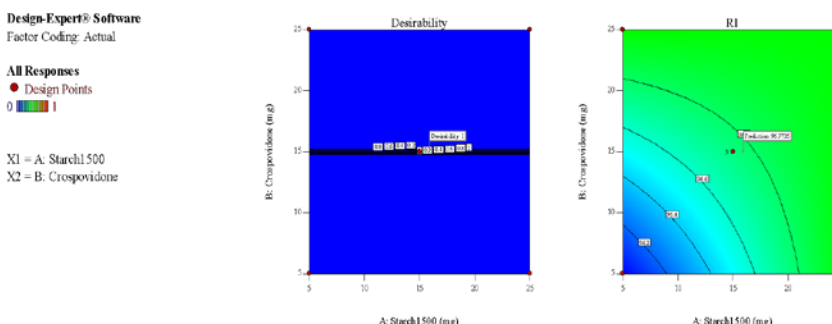


Fig. 6: Contour plot of the formulation variables on dissolution (Layer-II)

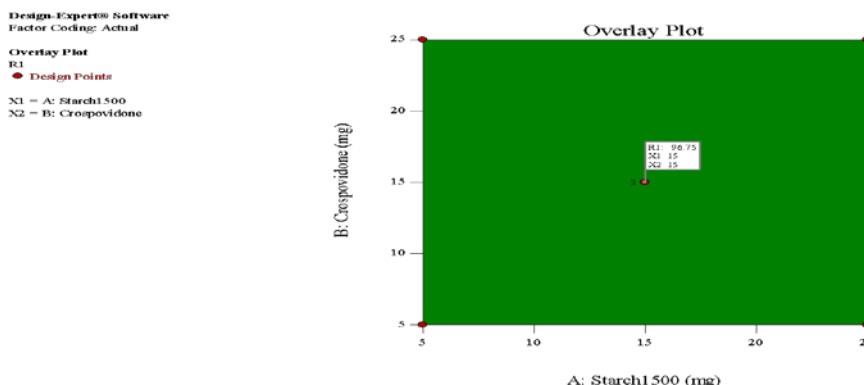


Fig. 7: Overlay plot-effect of formulation variables on responses (Layer-II)

Hot-melt extrusion process optimization of layer-I (ritonavir part)

The hot-melt extrusion process is a critical step in the manufacturing of robust products and needs optimization of the process. Center point batches of ritonavir target formulation (F12, F17 and F18) as per table 3 were considered for the process optimization. Risk assessment of the manufacturing process was carried out and the design of experiments (DOE) was designed for the parameters as mentioned in table 9.

Ritonavir part process variables in hot-melt extrusion like temperature, feed rate, and screw rotate per minute (RPM) were studied in higher and lower levels, including center points to evaluate related substance effect on drug effect. For the design of experiments (DOE) interpretations of related substances, only the highest unknown impurity levels were considered as a response as the other impurities were observed well within the limit. Hence the impurities other than the highest unknown are considered as low risk.

Table 9: Variables (Levels and constraints) for ritonavir hot-melt extrusion process

Independent variables	Different levels		
	Low	Medium	High
Temperature (C)	105	120	135
Feed rate (g/min)	20	25	30
Screw speed (RPM)	125	175	225
Response	Goal	Acceptable Range	
Maximum unknown impurity	Minimize	NMT0.200%	
Diss. in 0.025 N HCL+0.06 MPOE10LE	Maximize	NLT85 (Q)at 120 min.	

In the hot-melt extrusion process, the variation in temperature, feeding rate, and screw speed have an impact on the degradation of the product. Which indirectly affects the assay. Hence the risk on the assay and related substances is high. Solid dispersion takes place in a hot-melt extrusion process and it increases the dissolution of the drug product. The hot-melt extrusion (HME) parameters such as temperature, screw rotate per minute (RPM), and feeding rate have an impact on solid dispersion. Hence, the risk is high. All the responses were fitted to linear, interaction, or quadratic models using design expert version.11. Stat-Ease

Minneapolis USA and analyzed statistically using analysis of variance (ANOVA) and those are having a p-value less than 0.05 were included in the analysis. The data were also subjected to 3-D response surface methodology to study the interaction of process parameters and their effects on dependent variables. Thus, from the design of experiments (DOE) data, the design space was constructed within the acceptable limit. Hence, full factorial design, 2 level, three factors with 3 center points were selected for the design of experiments (DOE) evaluation of the hot-melt extrusion process as per table 10.

Table 10: Factors and responses for the ritonavir hot-melt extrusion (HME) process optimization

Batch No.	Std.	Run	Factor 1 A: temperature (C)	Factor 2 B: feed rate (g/min)	Factor 3 C: screw speed (RPM)	Response 1 RS (%): highest unknown limit: NMT 0.200%	Response 2 dissolutions at 120 min limit: NLT85(Q) at 120 min*
F26	10	1	120	25	175	0.077	99±0.59
F27	5	2	105	20	225	0.111	95±0.85
F28	9	3	120	25	175	0.063	99±0.77
F29	4	4	135	30	125	0.195	96±0.74
F30	8	5	135	30	225	0.19	99±0.98
F31	6	6	135	20	225	0.186	101±0.68
F32	2	7	135	20	125	0.204	100±0.75
F33	11	8	120	25	175	0.063	98±1.40
F34	1	9	105	20	125	0.045	99±0.96
F35	3	10	105	30	125	0.07	101±0.85
F36	7	11	105	30	225	0.045	100±0.88

*Data given in mean±SD

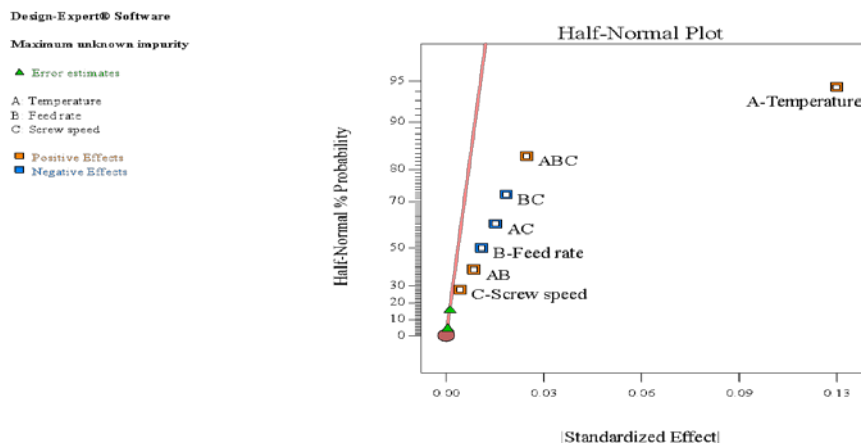


Fig. 8: The half-normal plot of the hot-melt extrusion (HME) process variable effects on related substances (Layer-I)

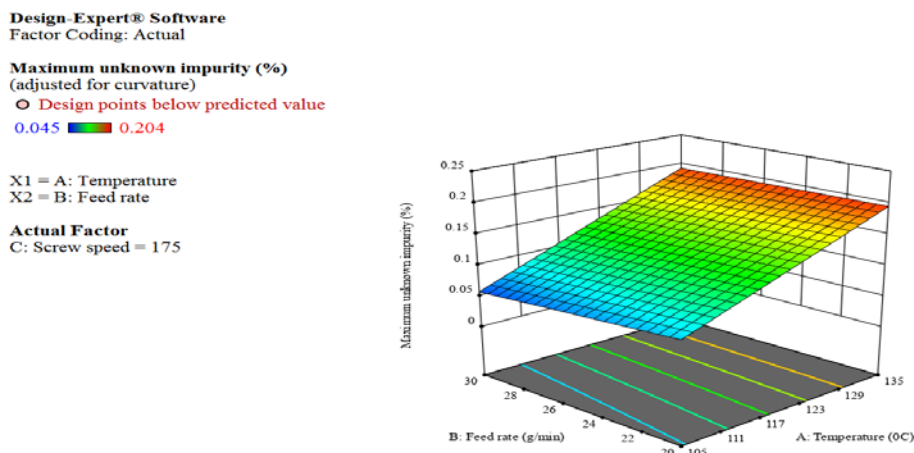


Fig. 9: 3D surface plot of the hot-melt extrusion (HME) process variable effects on related substances (Layer-I)

Table 11: Factors and response of design of experiments (DOE) for the ritonavir hot-melt extrusion (HME) process optimization

Response	Source	Sum of squares	df	Mean square	F value	P-value*	Comments
RS (%):	Model	0.0348	7	0.0050	76.19	0.0130	Significant
Highest	A-Temperature	0.0318	1	0.0318	486.00	0.0021	Significant
unknown	B-Feed rate	0.0003	1	0.0003	4.05	0.1819	Not significant
impurity Limit:	C-Screw speed	0.0000	1	0.0000	0.6199	0.5136	Not significant
NMT0.200%	AB	0.0002	1	0.0002	2.48	0.2560	Not significant
	AC	0.0005	1	0.0005	7.84	0.1074	Not significant
	BC	0.0008	1	0.0008	11.64	0.0762	Not significant
	ABC	0.0014	1	0.0014	20.69	0.0551	Not significant
	Curvature	0.0087	1	0.0087	132.90	0.0574	Not significant
	Pure error	0.0001	2	0.0001	-	-	-
	Total	0.0137	10	-	-	-	-

*P-value less than 0.0500 indicate above model terms and factor-A are significant

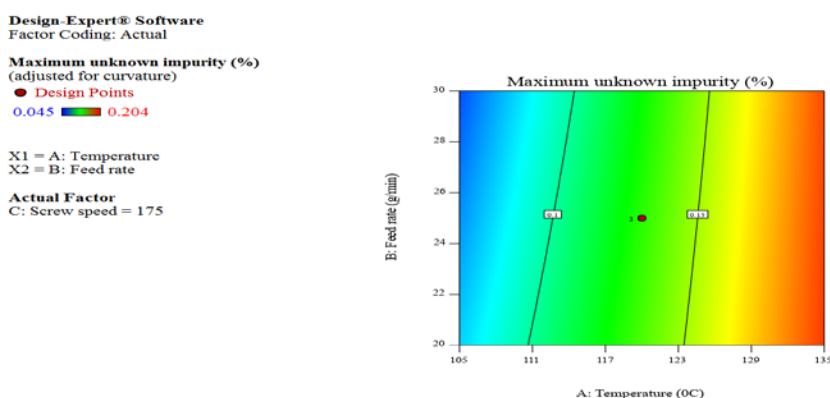


Fig. 10: Contour plot of the hot-melt extrusion (HME) process variable effects on related substances (Layer-I)

Table 12: Factors and response of design of experiments (DOE) design for the ritonavir hot-melt extrusion process

Response	Source	Sum of squares	df	Mean square	F value	P-value*	Comments
Diss. in 0.025 N	Model	34.88	7	4.98	14.95	0.0641	not significant
HCL+	A-Temperature	0.1250	1	0.1250	0.3750	0.6026	not significant
0.06 M	B-Feed rate	0.1250	1	0.1250	0.3750	0.6026	not significant
POE	C-Screw speed	0.1250	1	0.1250	0.3750	0.6026	not significant
10LE	AB	21.13	1	21.13	63.38	0.0154	Significant
Limit:	AC	10.12	1	10.12	30.37	0.0314	Significant
NLT 80(Q)	BC	3.12	1	3.12	9.37	0.0922	not significant
At 120 min	ABC	0.1250	1	0.1250	0.3750	0.6026	not significant
	Curvature	0.0947	1	0.0947	0.2841	0.6473	not significant
	Pure Error	0.6667	2	0.3333	-	-	-
	Cor total	35.64	10	-	-	-	-

* P-value less than 0.0500 indicate above model terms and interaction factors-AB and AC are significant

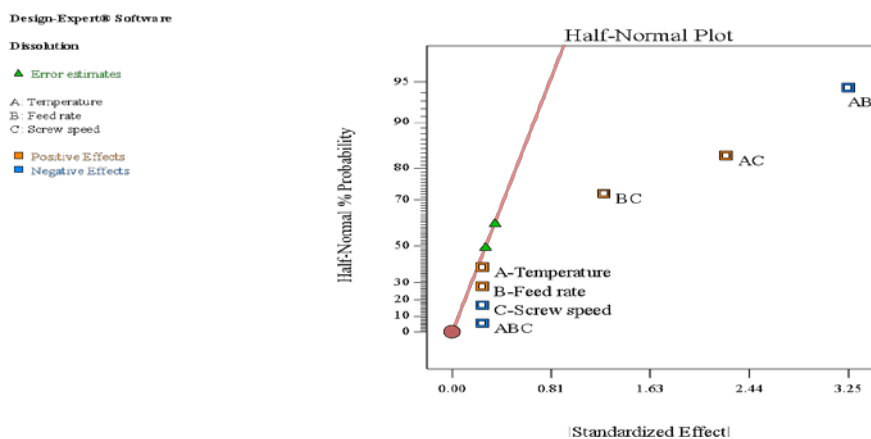


Fig. 11: Half normal plot of the hot-melt extrusion (HME) process variable effects on dissolution (Layer-I)

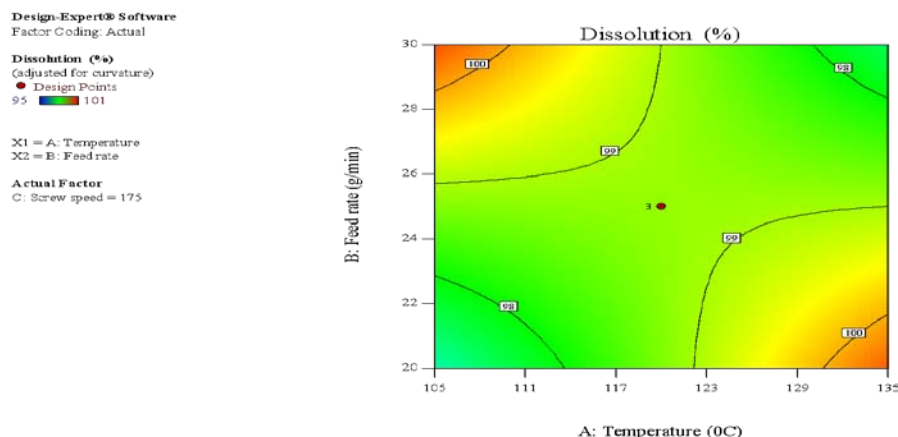


Fig. 12: Contour plot of the hot-melt extrusion (HME) process variable effects on dissolution (Layer-I)

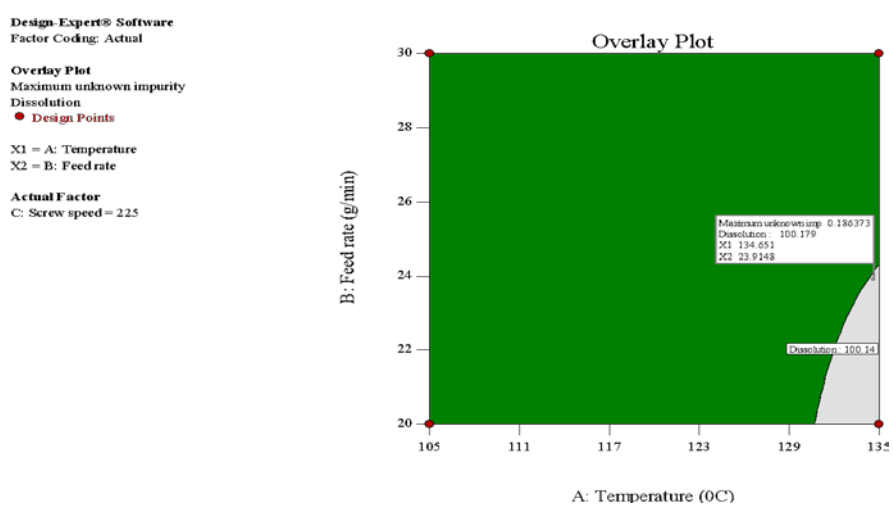


Fig. 13: Overlay plot of the hot-melt extrusion (HME) process variable effects on dissolution and related substances (Layer-I)

Based on the evaluation of analysis of variance (ANOVA) responses, the highest unknown impurity observed to be failure meets the specification with Factor-A (Temperature) during the extrusion process. As per the half-normal plot, 3D surface plot, and contour plot, temperature plays an important role in controlling the unknown impurities within the limit. Temperature above 130 °C along with feeder rate from 20-24 (g/min) has been removed from the design of experiments (DOE) range and it was found to be a failure to meet the maximum unknown impurity limit.

As per the design of experiments (DOE) studies, these three factors had no significant impact on the dissolution of finished products even though interaction factors AB and AC showed significance in the Analysis of variance (ANOVA). As per the above overlay plot, the green zone indicates all the responses meet the specification limits well within the limit. Grey zone indicates a failure to meet the specification limits. Because no curvature effects were observed for any of the responses studied using full factorial design of experiments (DOE), further studies to optimize the ritonavir part process were unnecessary.

Based on the process design of experiments (DOE) study for the ritonavir part, a feed rate of 25-30 g/min and a temperature range from 105-130 °C can be recommended to control the responses of maximum unknown impurity and dissolution results well within the limit.

Wet granulation process optimization of Layer-II (Atazanavir sulfate)

The formulation design for Layer-II by wet granulation with purified water used as the granulating solvent. Granulation process parameters

such as binder addition time, fluid uptake, impeller and chopper speed, kneading time, wet milling, and dry milling screen size, and mill speed are considered as critical parameters as part of the risk assessment for the granulation process. But, all these parameters have no significant impact on the dissolution at 15 min and it was observed more than 85% for all the batches in the recommended dissolution media. However, fluid uptake, impeller speed, and kneading time resulted to be variation for dissolution at 5 min and 10 min dissolution comparatively with target granulation process parameters. The wet granulation process has no significance on the dissolution of the drug product in the selected dissolution media at 15 min. Hence, process parameters were used for the wet granulation process for Layer-II were not considered for the optimization study.

The factorial batches were prepared using the design of experiments (DOE) for the hot-melt extrusion process for tablet properties observed to comply with the acceptable limits. Parle elizabeth with a lab model of a bilayer compression machine was used for the compression. Ritonavir part (layer-I) was used as the first layer and compression parameters were set as per table 13. After setting the first layer, atazanavir sulfate (layer-II) was loaded into the hopper for second layer compression, and other compression parameters such as feeder speed, fill depth, pre-compression force, main compression force was maintained for the optimum physical parameters such as hardness, thickness, disintegration time, friability, and physical appearance.

As per table 13, design of experiments (DOE) batches from F026 to F36, all the physical parameters are found to comply during the execution of compression. In which disintegration time for layer-I

and layer-II were monitored and layer-I was observed about 5-7 min and layer-II was observed around 29-36 min. All the studied batches for dissolution were found to comply well within the acceptable limits and comparable with each reference product. Based on the

evaluation of dissolution results in table 10, there are no significant differences among all the batches and consistent within the studied hardness range of 16-28 N at the recommended dissolution time point of 120 min.

Table 13: Compression parameters and physical parameters of the core tablets

Parameters *		F26	F27	F28	F29	F30	F31	F32	F33	F34	F35	F36
Turret speed		10										
Feeder speed (RPM)	S1	7 (Ritonavir part)										
	S2	5 (Atazanavir sulfate part)										
Fill depth (mm)	S1	5.15±0.5	5.15±0.3	5.17±0.8	5.21±0.7	5.17±0.8	5.17±0.4	5.16±0.7	5.15±0.4	5.19±0.6	5.14±0.6	5.15±0.9
	S2	11.90±0.3	11.98±0.5	12.10±0.6	11.78±0.2	12.10±0.3	11.98±0.6	11.98±0.6	11.90±0.8	11.96±0.6	11.98±0.8	12.46±0.2
PCF position (mm)	S1	3.2	3.3	3.4	3.6	3.3	3.4	3.6	3.2	3.2	3.3	3.2
	S2	4.9	4.5	4.8	4.6	4.8	5.1	5.2	4.9	4.9	4.8	5.2
MCF position (mm)	S1	8.4	8.4	8.5	8.9	8.6	8.2	8.2	8.4	8.2	8.3	9.8
	S2	4.0	4.2	4.3	4.2	4.2	3.9	4.9	4.1	4.1	4.2	4.6
Average force of PCF(kN)	S1	3.10	3.12	3.80	3.16	3.14	3.16	3.20	3.10	3.60	4.10	3.70
	S2	4.10	4.60	4.20	4.30	4.28	4.50	4.18	4.10	4.10	4.20	5.80
Average force of MCF(kN)	S1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S2	16.05±0.25	17.05±1.25	16.35±0.95	16.71±0.71	16.05±0.85	16.45±2.65	16.35±0.55	16.05±0.25	18.05±1.25	17.05±2.25	18.21±0.40
Weight variance	Avg	1605.0±1.20	1602.0±1.62	1596.0±0.43	1610.0±1.24	1604.0±2.24	1611.0±1.82	1590.0±1.42	1601.0±1.30	1594.0±0.82	1600.0±2.48	1602.0±1.99
Hardness range (kP)	16-28kP	20.90±1.95	20.90±2.0	20.0±2.2	21.20±2.80	22.00±1.8	20.70±2.1	21.00±3.0	21.00±2.2	21.00±2.85	20.00±2.2	23.00±1.8
Thickness (mm)	8.00-8.60	8.34±0.1	8.32±0.2	8.34±0.1	8.28±0.2	8.31±0.2	8.36±0.1	8.32±0.2	8.31±0.2	8.32±0.2	8.34±0.2	8.28±0.2
Disintegration time (min/sec)	Atazanavir:	6 min 15 sec	6 min 15 sec	6 min 50 sec	5 min 55 sec	6 min 45 sec	6 min 20 sec	6 min 15 sec	6 min 15 sec	6 min 15 sec	6 min 15 sec	7 min 55 sec
	NMT15 min											
	Ritona vir:	35 min 20 sec	38 min 20 sec	32 min 20 sec	34 min 20 sec	36 min 20 sec	29 min 20 sec	35 min 20 sec	35 min 20 sec	35 min 20 sec	35 min 15 sec	36 min 20 sec
	NMT60 min											
Friability	NMT1.0%	0.4±0.01	0.4±0.02	0.4±0.01	0.4±0.02	0.4±0.02	0.4±0.02	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.02	0.4±0.01

*Data given in mean±SD, Based on the evaluation of formulation and process optimization, the final formulation in table 14 was recommended for stability.

Tablet 14: Proposed formulation for stability studies

S. No.	Ingredients	Mg/tab
1	Atazanavir sulfate	341.7
2	Lactose monohydrate	152.0
3	Starch 1500	15.0
4	Crospovidone	15.0
5	water	Q. S
Extra granular		
5	Starch 1500	50.0
6	Crospovidone	50.0
7	Calcium silicate	40.0
8	Ferric oxide yellow	0.5
9	Magnesium stearate	5.8
Total weight of Atazanavir layer		670.0
10	Ritonavir	100.0
11	Co povidone	585.0
12	Sorbitan monolaurate	60.0
13	Colloidal silicon dioxide	8.0
Extra granular		
14	Dicalcium phosphate anhydrous	110.0
15	Colloidal silicon dioxide	20.0
16	Sodium stearyl fumarate	7.0
Total weight of ritonavir layer		890.0
Total weight of core tab		1560.0
17	Opadry II yellow	40.00
18	Purified water	Q. S
Total weight of coated tab		1600.00

Drug release is usually the rate-limiting process for absorption of the biopharmaceutical classification system (BCS) class II and class IV compounds due to their low solubility. Atazanavir sulfate is a biopharmaceutical classification system (BCS) class II, drug and ritonavir is a class IV drug. Therefore, the dissolution of the reference product was compared with a fixed-dose formulation in the in-house dissolution media of 0.025 NHCL+0.06 MPOE10LE, volume: 900 ml apparatus type I, agitation speed 100 rotate per minute (RPM). Dissolution media was selected based on patent research data and the solubility of both active substances such as ritonavir and atazanavir sulfate.

As per fig. 14, the formulation study of batches from F15 and F16 contains 40 mg of span 20 with aerosil and without aerosil and the dissolution rate resulted as low. Batches from F12 and F13 contain 80 mg of span 20 with aerosil and without aerosil indicates a high dissolution rate. But, center point batches of F12, F17, and F18 results were found to be optimum and comparable with the reference drugs. As per fig. 15, the formulation study of batches from F19 to F25 contains starch and crospovidone studied range has no significant impact on the dissolution at 15 min and drug release observed more than 85 %. But, F19 and F20 contain 5 mg of crospovidone with 5 mg and 25 mg of starch indicates there is a variation of dissolution rate 5 min and 10 min. In addition, batches from F21 and F22 contain 25 mg of crospovidone with 5 mg and 25

mg of starch resulted to be higher dissolution rate comparatively with center point batches and reference drugs. Based on the evaluation of the design of experiments, surfactant (span 20 mg) with aerosil and without aerosil is very critical in the ritonavir formulation. Disintegrant such as crospovidone with binder such as starch 1500 and without starch 1500 is no critical in the atazanavir formulation. Hence, the optimum formulation was manufactured with two layers separately, such as S1layer (ritonavir) and S2layer (atazanavir sulfate) up to the final blend. Both blends are compressed by using bilayer Compression. Further, the impact of hardness was evaluated on the dissolution of both layers. As per fig. 16, low hardness at 16kp has faster dissolution and high hardness at 28kp has slower dissolution. Overall, the studied hardness range has no significant impact on both layer. There is a variation of dissolution rate at initial time points and the studied hardness range comparable with the reference drug product. Bilayer compression followed by film coating was executed with opadry yellow of hydroxypropyl methylcellulose (HPMC) based coating with a solid concentration of 10%w/w and weight build-up was targeted for the 2.53 % for each formulation. As per fig. 17, the finished product of fixed-dose formulation of dissolution results is comparable with each reference product of the innovator product. So, fixed dose of test formulation yielded the results of content uniformity, related substances, and physical appearance of finished product same as that of the reference product.

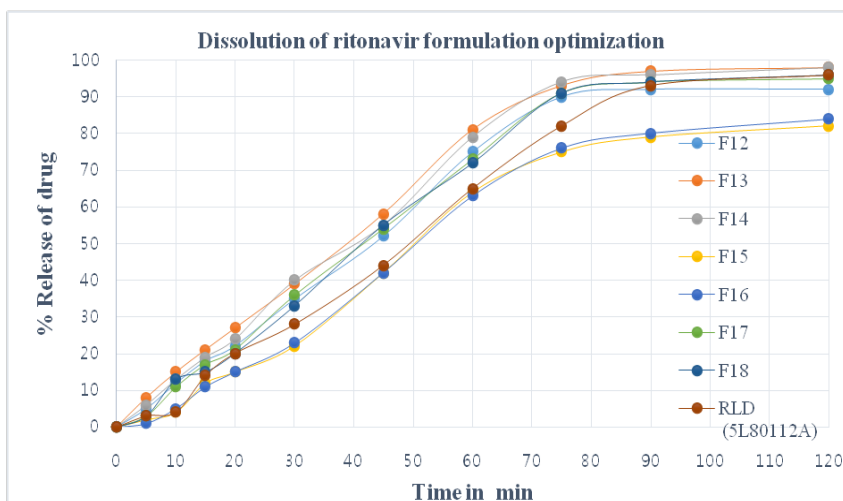


Fig. 14: Dissolution of ritonavir formulation optimization

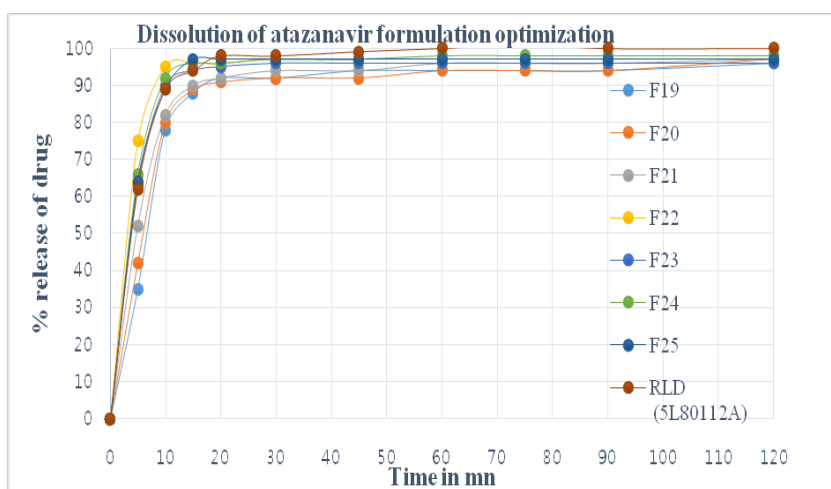


Fig. 15: Dissolution of atazanavir formulation optimization

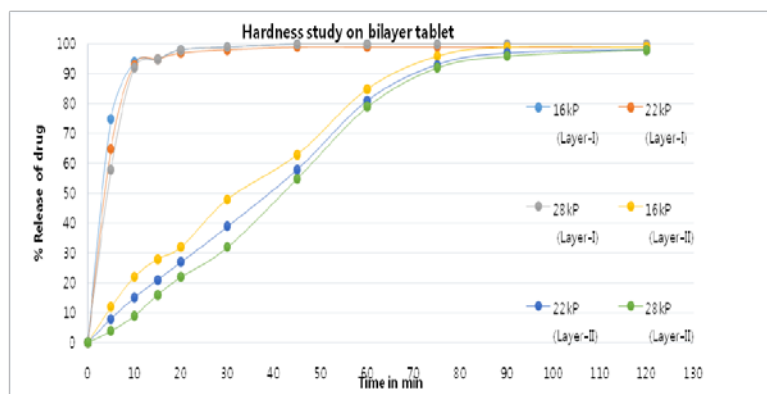


Fig. 16: Dissolution of hardness study on bilayer tablet

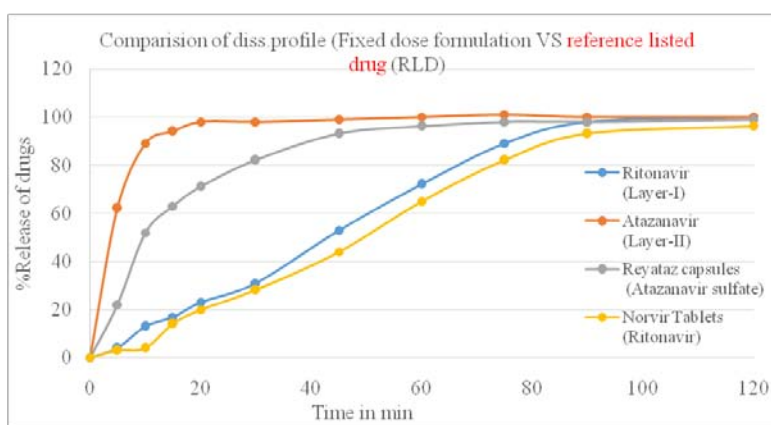


Fig. 17: Comparison of dissolution profile for fixed-dose formulation (test) vs reference product

DISCUSSION

Pre formulation study and drug-excipients compatibility study was carried out as per the international conference of harmonization (ICH) guidance. Bilayer formulation was designed for a fixed-dose combination of ritonavir and atazanavir sulfate as per the research and patents data [11-14]. Further, dissolution method development was established based on the solubility studies of both active substances [17, 18].

Initially, atazanavir formulation was designed by using direct compression [9] and resulted in poor flowability and content uniformity issues were observed. Further, the wet granulation method was suggested to improve the flowability of the final blend [16]. Hence, diluent, binder, and disintegrant were selected based on the literature, and the studied range was fixed based on the handbook of excipients [20]. Finally, the formulation was optimized through the design of experiments (DOE) by using starch and croscopovidone with different levels. Analysis of variance (ANOVA) for the model also shows significant. But, the studied range of starch and croscopovidone from 10.00 mg to 25.00 mg has no significant impact on the dissolution because of the maximum drug was released within 85 % at 15 min in the recommended dissolution media. Counter plot and overlay plot also suggest that the studied formulation of starch and croscopovidone has no significant impact on the dissolution [21, 22]. Further, wet granulation improved the content uniformity and there is no interaction with the ritonavir layer.

In the case of ritonavir formulation, risk assessment was carried out for the formulation and process part as per the quality by design (QbD). Sodium lauryl sulfate (40.00-80.00 mg) and colloidal silicon dioxide (0.00-16.00 mg) range were selected to study the impact on the dissolution through the design of experiments [12-14]. Analysis of variance (ANOVA) for the model and factor-A in the formulation has a significant impact on the dissolution. Half-normal plot and

counterplot indicates that sodium lauryl sulfate range has a significant impact on the dissolution and overlay plot revealed that lower range of sodium lauryl sulfate failure to meet the specification limit. Further, the hot-melt extrusion process of the ritonavir part, temperature, feed rate and screw speed was selected for the study-related substances. Analysis of variance (ANOVA) for the model, half normal plot for temperature shows significant on related substances and there is no impact on the dissolution. However, the 3D surface plot and counterplot indicates studied temperature range may impact on the related substances. But, the interaction of AB and AC factors shows significant as per the analysis of variance (ANOVA) and there is no evidence of impact on the dissolution of tablets. Finally, the overlay plot represents higher levels of temperature range impact on the related substances of finished product and failure to meet the specification [21, 22]. So, the design of experiments through full factorial and fractional factorial design resulted to find the formulation and process range to control the dissolution and unknown impurities well within the limit. Further, all the excipients quantities were used for both layers of formulation followed to be within the inactive ingredients (IID) list as per the united states food drug administration (USFDA) [23].

CONCLUSION

Fixed-dose formulation of ritonavir and atazanavir sulfate tablets 100 mg was successfully manufactured with various percentages of diluents, solubilizers, polymers, and Disintegrants and finalized the stable formulation. Based on the solubility studies of active substances, a dissolution method was established. Compatibility studies were carried out for both mixtures of final blend formulations before the bilayer tablet compression for the safety of optimum formulation. Based on these studies confirmed that there is no interaction between both layers. Fractional factorial design and full factorial design were evaluated to understand the critical impact of formulation and process factors on the finished product. Design

space was recommended for consistent results of the finished product. Further, the final product of fixed-dose formulation of ritonavir and atazanavir sulfate tablets 100 mg/300 mg complies with the dissolution results with each formulation of atazanavir tablets 300 mg and ritonavir capsules 100 mg. Overall the effect of fixed-dose formulation has synergetic than individual dosage forms of each tablet and capsule. It can be concluded that fixed-dose formulation available as a single dosage regimen and shows better *in vitro* drug release comparatively with innovator formulations and related substances observed to be well within the acceptable limit as per the international conference on harmonization (ICH) guidelines. Hence, the final formulation was stable and robust.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of the paper.

REFERENCES

1. Arun K Tiwari, Pawan K Porwal. Fixed-dose combination product: current status among regulatory. Res J Pharm Tech 2009;2:433-8.
2. Bangalore, S Shahane, A Parkar. Compliance and fixed-dose combination therapy. Curr Sci Inc 2007;9:184-9.
3. Jagannath Kota, Phaninatha Sarma Ayalavajjala. Development of orally administered fixed-dose combination (FDC) products: pharmacokinetic and biopharmaceutical considerations. Int J Sci Res 2015;3:161-73.
4. Shein Chung Chow. Bioavailability and bioequivalence in drug development. Wiley Int Rev Comput Stat 2014;6:304-12.
5. Dighe PP, T Hm. Design and statistical optimization of a bi layered tablet of metoprolol succinate sustained-release and atorvastatin calcium immediate release: once as day formulation in the management of hypertension. Asian J Pharm Clin Res 2018;11:293-302.
6. Pearl Pires Dighe, HM Tank. Formulation development and statistical optimization of as bilayer tablet of bosentan monohydrate and sildenafil citrate in management of pulmonary arterial hypertension. Int J Appl Pharm 2019;11:2.
7. Vishwakarma AG, Mogal RT, Pawar AY. Bi-layer tablet-a new ways in oral drug delivery system. Int J Pharm Tech Res 2014;6:1416-28.
8. International Conference of Harmonization (ICH). Harmonized tripartite guidelines for stability testing of new drug substances and products Q1A (R2). Rockville, MD: United States Pharmacopoeial Convention, Inc; 2003. p. 6.
9. Jampala Raj Kumar, P Narayana Raju, GV Radha, Aligeti Sravan Kumar. Design and evaluation of atazanavir sulfate non-effervescent sustained release floating matrix tablets. Global J Pharm Pharm Sci 2019;7:1-12.
10. K Kavya, P Sobhita Rani, A Pavani, E Rajareddy. Formulation and evaluation of immediate-release tablets of atazanavir. Int J Pharm Sci Rev Res 2014;24:119-23.
11. Patents. google.com/patent/US20120121722A1/en
12. Desai S, J Disouza, K Musle, H Avinash. Solubility enhancement of ritonavir by hot-melt extrusion. Int J Pharm Sci 2016;8:309-12.
13. MA Repka, S Shah, J LU. Melt extrusion process to product. Expert Opinion Drug Delivery 2012;9:105-25.
14. Patents. google.com/patent/US20150045400A1/en
15. https://www.ema.europa.eu/en/documents/product-information/ritonavir-mylan-epar-product-information_en.pdf. [Last accessed on 10 Mar 2021]
16. https://www.ema.europa.eu/en/documents/assessment-report/atazanavir-mylan-epar-public-assessment-report_en.pdf. [Last accessed on 10 Mar 2021]
17. <https://extranet.who.int/pqweb/sites/default/files/HA632par t6.pdf>. [Last accessed on 10 Mar 2021]
18. https://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_getalldata.cfm. [Last accessed on 10 Mar 2021]
19. Niraj S, Trasi, Sonal bhujbal, Qi Tony Zhou, Lynne S Taylor. Amorphous solid dispersion formation via solvent granulation-a case study with ritonavir and lopinavir. Int J Pharm 2019;X1:100035.
20. Handbook of pharmaceutical excipients. 9th edition. edited by Paul J Sheskey, Brune C Hancock, Gary P Moss, David J Goldfarb; 2020.
21. Hemant K Jain, Madhuri Taware. Formulation development and evaluation of gastro-retentive dosage form of atazanavir sulfate. Int J Pharm Sci 2018;10:60.
22. www.statease.com (DESIGN-EXPERT® VERSION 11 software). [Last accessed on 10 Mar 2021]
23. <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm> [Last accessed on 10 Mar 2021]