Original Article

Cocrystals Of Lopinavir: Effect On Drug Release And Hygroscopicity Characteristics

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Abstract

Lopinavir is a BCS class II antiretroviral drug with mild hygroscopic characteristics necessitating approaches to improve dissolution and control the humidity. Cocrystals offer a suitable approach to improve dissolution and modify handling properties of substances in favourable manner. The Lopinavir was cocrystallized by solvent evaporation liquid assisted grinding and ultrasonic assisted cocrystalization. Highest drug release was seen in cocrystals prepared by solvent evaporation. The drug release was improved by twice and it was seen that ex vivo absorption also increased 1.56 times. Moisture uptake of cocrystals drastically reduced as compared with lopinavir.

Keywords: Cocrystal, Lopinavir, hygroscopic, ex vivo, dissolution

INTRODUCTION:

Hygroscopicity is the tendency of a solid substance to absorb moisture from the surrounding atmosphere. The process can take on a number of forms. Thus, with a porous solid such as activated carbon, water vapor will be physically adsorbed, both on the external surface and within the pores, to form a condensed layer.[1]

Hygroscopicity poses challenges in formulation such as poor flow, microbial growth, degradation and content variation in dosage forms. The API may undergo degradation processes through hydrolysis.

Crystallographic phase changes in the drug can happen during storage at low or high relative humidity affecting its bioavailability adversely.

Second, moisture may bring on phase transitions by lowering glass transition temperatures and acting as a plasticizer in amorphous solids It may induce recrystallization of intended amorphous forms, which were formed for their enhanced solubility, back into stable crystalline forms with decreased solubility and bioavailability
This necessitates processing of hygroscopic material under controlled humidity environment and use of special packaging matrials to protect them.

Instead of these cost enhancing and inadequate measures it is necessary to pre-empt these problems using formulation approaches such as coating, encapsulation, cocrystallization etc.

Co-crystals are defined as “solids that are crystalline single-phase materials composed of two or more different molecular and/or ionic compounds (co-former) generally in a stoichiometric ratio which are neither solvates nor simple salts”. These offer a relatively simple strategy for improvement of dissolution, bioavailability, stability, solubility and hygroscopicity of compounds.[6]

Selection of suitable conformer is important to the properties of cocrystals formed. Various approaches to coformer selection were supramolecular synthon approach, Hansen solubility, parameter, pKa based, hydrogen bond propensity, Cambridge structural database and Molecular docking[4]

Lopinavir is an antiretroviral protease inhibitor used in combination with ritonavir in the therapy and prevention of HIV and AIDS. It is a BCS class II drug with LogP 5.94 Lopinavir is Moderately hygroscopic.[5]

Present study describes studies involving conformer selection for making lopinavir cocrystals, effect of stoichiometry and characterisation of co-crystals of Lopinavir. The cocrystals are formulated as tablets and evaluated for their moisture uptake.

• MATERIALS AND METHODS:

Material:

Lopinavir was received as a gift sample from Glaxo Smithkline. All required solvents and excipients were provided by LOBA CHEMIE PVT LTD. Molecular docking was performed on Schrodinger suit version 9.0 software. Experimental design was performed using design expert version 12.0 software.

METHOD:

Solubility parameter:

Solubility parameter was calculated by group contribution method of Fedor.

Preparation of lopinavir cocrystal:

Method of preparation

1. Solvent Evaporation Method:

A specific quantity of lopinavir and coformer (Molar mass of lopinavir and coformer was taken in 1:1, 1:2 and 2:1 ratios) was weighed. The powdered mass then transferred to a glass beaker and dissolved in methanol with constant stirring. The stirring was continued until a clear solution is obtained. The solution then filtered through a filter paper and the solvent was evaporated at room temperature until a dry mass is obtained. And then screened with sieve of mesh size 60 and stored in airtight container.

2. Liquid assisted grinding:

A specific quantity of lopinavir and coformer was weighed and ground with mortar pestle for 10 minutes. Then small quantity of methanol was added to form a slurry like consistency. After 30 minutes of grinding the slurry was transferred to the petri dish and let it to dry at room temperature to get dry mass. The mass then screened with sieve of mesh size 60 and stored in airtight container.

3. Ultrasonic assisted cocrystallization:

Lopinavir and coformer were dissolved together in methanol. The clear solution was subjected to sonication using solid probe of probe sonicator (Make Sonic Model VCX 750). Stop the sonication when the solution becomes turbid. Then filter it. And dry it overnight to get the cocrystal.

CHARACTERIZATION OF COCRYSTAL

1. Differential scanning calorimetry:

The DSC thermogram of lopinavir and co-crystal was recorded on Mettler Toledo 823. The samples were heated in 40 µl aluminium crucibles from 30° to 300°C at a heating rate of 10°C/min under nitrogen atmosphere.

2. Infra-red Spectroscopy

The prepared cocrystals were characterised using infrared spectroscopy. The spectra were collected over the range of 4000-600 cm^{-1} in 32 scans, with resolution of 4 cm^{-1} for each sample (Jasco FTIR 4100).

3. In-vitro drug release:

Dissolution test (n=3) of Co-crystal and lopinavir was carried out in 2% sodium lauryl sulphate (900 ml, 37±0.5°C, 50 RPM) for 60 min using USP II paddle apparatus. At predetermined time intervals, 10 ml samples were withdrawn and replaced with equal volume of fresh dissolution medium. The dissolved drug concentration in aliquots collected at different time intervals were analysed by UV spectrophotometry at 240 nm.

4. Solubility study:

Solubility of lopinavir and lopinavir cocrystals was tested in distilled water by shake-flask method. Excess quantity of lopinavir and equivalent cocrystals was added separately to 250ml of conical flask containing 100 ml of distilled water. Flask was shaken by orbital shaker (REMI Instruments CIS-24BL) with stirring speed 150 RPM at temperature 37±0.5°C for 12 hours. Filtered through (0.45µm filter) concentration was determined by UV spectrophotometry. Test was conducted with three repetitions

5. Ex vivo permeation study:

The ex vivo permeation of dissolved drug was measured using everted rat intestine model. The 8 cm intestinal segment was obtained from sacrificed rat and was washed carefully using Krebs ringer solution. This was everted over glass rod and was stored in Ringer solution oxygenated with O_2/CO_2 (95%/5%) at 37^0C. The ex vivo permeation was measured along with the invitro dissolution study described above by inserting apparatus in the dissolution vessel. In this system drug diffusion from formulation and permeation across everted intestine occurred simultaneously. The permeation of lopinavir from lopinavir containing vessel and from lopinavir cocrystals containing vessel was measured by collecting

aliquots at pre-determined time intervals of 0, 10, 20, 30, 40, 45min. The sample was analysed on UV spectrophotometer at 240 nm wavelength

Evaluation of micromeritics property

The micromeritic properties of lopinavir and lopinavir co-crystals were evaluated through measurement of bulk density, angle of repose, Hausner's ratio and carr's index as described in literature.

Preparation of tablets

Lopinavir co-crystal, was converted to tablet, excipients used were Sodium starch glycolate as super disintegrant, microcrystalline cellulose as binder, Lactose as diluent, magnesium stearate as lubricant and talc as glidant. The mass was mixed with PVP K30 and Silicon dioxide. Compressed using 8 mm punch using B tooling on Rimek MINI PRESS-II MT.

Experimental Design

Experimental Design 3^2 full factorial design was used to evaluate two variables at 3 levels viz. Lactose: MCC in ratio (90:10, 80:20, 70:30 mg) and concentration of Sodium starch glycolate (10, 15, 20 mg) in order to determine their hardness, friability and % drug release (table 1). The layout of experimental design is shown in table 6. Two factors were evaluated each at three levels & experimental trials were performed at all possible nine combinations as shown in table 6.

Evaluation of tablets: The tablets were evaluated for attributes such as hardness, friability, uniformity of weight as per methods in literature. The invitro dissolution rate study was performed.

Table 1: 3^2 full factorial design for optimization lopinavir and lopinavir- cinnamic acid co-crystal tablet

Sr no	Factors				Response
1	Lactose: MCC	-1	0	1	Hardness, friability and percentage drug release
2	SSG	-1	0	1	

Table 2: Composition of lopinavir- cinnamic acid co-crystal tablet (mg)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lopinavir cocrystals	294	294	294	294	294	294	294	294	294
Lactose and MCC ratio	90:10	90:10	90:10	80:20	80:20	80:20	70:30	70:30	70:30
Sodium Starch Glycolate	10	15	20	10	15	20	10	15	20
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5
Total	414	419	424	414	419	424	414	419	424

Optimization and validation of model

The release data response was fed into the design expert software 12.0, which created the equations. The numerical optimization was carried out using the desirability function, and the projected formula was examined to see if the outcome matched DOE's optimized release data.

RESULT AND DISCUSSION

Solubility parameter

cohesive energies determine how a drug substance will behave when processed or when dosed in vivo. The most common approach in quantifying the cohesive energy for a drug substance is to determine its solubility parameter, d, which is defined as the square root of its cohesive energy density (CED), expressed as the energy of vaporisation per unit volume.

The solubility parameter is a numerical value that indicates the relative solvency behaviour of a specific solvent Some methods used to calculate solubility parameter are Small's method, Hoy's method, Van Krevelen's method, konstom and Fairhellar method, Rheineck method, Fedor's method. (table 3). Fedors' method was used in this work, it contains the contributions for a large number of functional groups and chemical structures. Out of various coformers lopinavir and cinnamic acid has closest solubility parameter values

Table no. 3: Solubility parameter

Sr no.	Name of drug/ coformer	Solubility parameter $(cal/cm^3)^{1/2}$	Difference
1	Lopinavir	10.8	-
2	Itaconic acid	11.8	-1
3	Stearic acid	8.3	2.5
4	Salicylic acid	12.5	-1.7
5	Succinic acid	11.9	-1.1
6	Malonic acid	13.1	-2.3
7	Benzoic acid	10.9	-0.1
8	Citric acid	14.1	-3.3
9	Fumaric acid	12.3	-1.5
10	Saccharine	15.3	-4.5
11	Glutaric acid	11.2	-0.4
12	Cinnamic acid	10.3	0.5
13	L arginine	13.2	-2.4
14	Nicotinamide	13.6	-2.8
15	Acetamide	12.4	-1.6
16	Urea	17	-6.2
17	Tartaric acid	12.7	-1.9

Preparation of cocrystals

Cocrystallization is the process of producing cocrystals, i.e., crystals with two or more molecular species in a specific stoichiometric ratio within a crystal lattice. The different molecular species associated with forming the cocrystal are referred to as the coformers. The coformer molecules are associated together primarily by noncovalent interactions such as hydrogen or halogen bonds

When two incongruently soluble components cocrystallize, the less soluble component precipitates preferentially, resulting in a solid mixture of cocrystal and cocrystal components or a failure to form cocrystals. Lopinavir and cinnamic acid are both slightly soluble in methanol have a good chance to precipitate together forming cocrystals.

The most common method for preparing cocrystals is solvent evaporation. Cocrystals formed by solvent evaporation have a lower energy and are more homogeneous in terms of crystal composition.

The method of preparation of cocrystals should provide required super saturation and molecular collisions to effect the uniform drug coformer distribution and molecular contact. Ultrasound-assisted cocrystallization is useful to obtain fine crystal size, while the cavitation energy of ultrasonic waves induces primary nucleation of particles and leads to supersaturation for cocrystal growth

1. Differential scanning calorimetry of lopinavir and Cocrystals

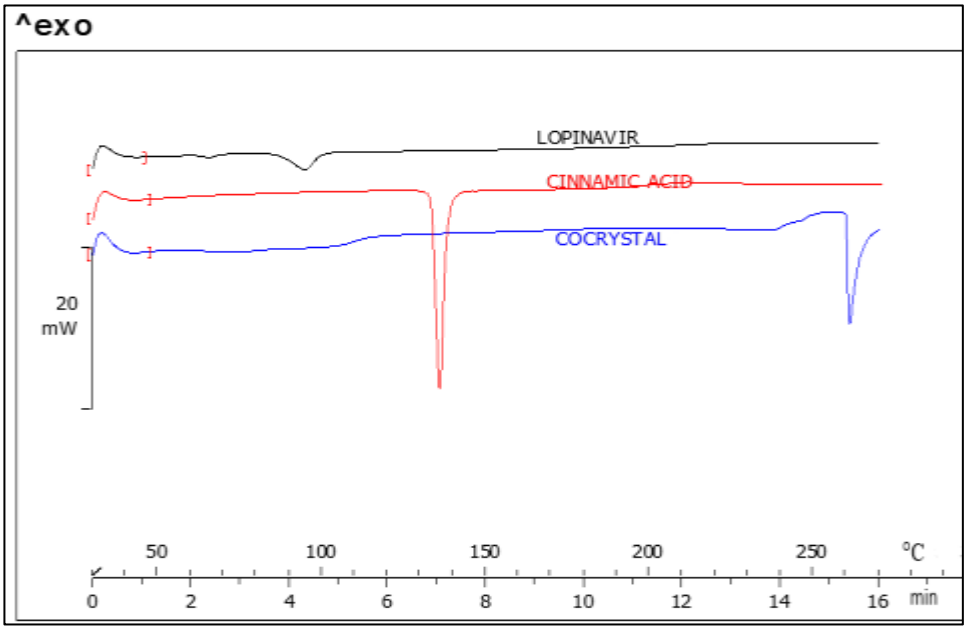

Figure 1: DSC thermogram of LOP and cocrystal

Melting point of lopinavir was observed 102°C while coformer cinnamic acid shows melting point at 133 °C. The formulated cocrystal endothermic peak was observed at 261°C which is higher than the pure drug (figure 20)

2. Infra-red spectroscopy of lopinavir-cinnamic acid cocrystals

The possible interaction between the drug and the coformers was studied by IR spectroscopy. From the results of IR, it was observed that all the important peaks due to functional groups of LOP were present the peak at 1527,1430,1610 cm^{-1} indicates benzene ring, peak at 1708.62 cm -1 indicates C=O stretching, whereas peaks at 1750cm -1 indicates CH2=CH2 stretching. Peak at 3527, 3448 cm-1 indicates NH stretching. CIN IR showed a characteristic peak C=C stretching at 1680 cm-1 and C=O peak at 1705.73 cm^{-1} .

However, some changes in the cocrystal IR spectrum were observed such as presence of peak OH stretch at 2610.18 cm^{-1} in prepared TEL cocrystals (figure 2) when compared to pure drug, thereby indicating that hydrogen bonding has occurred in the cocrystals.

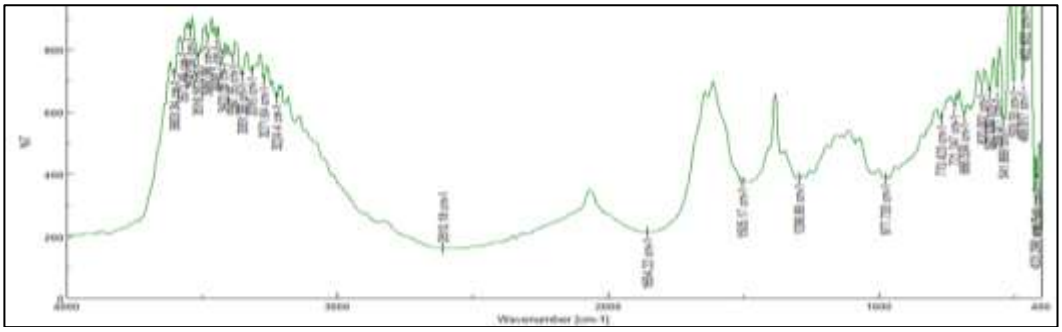

Figure 2: IR spectra of lopinavircocrystals

4. In vitro drug release from cocrystals

Table 4: Percent drug release for different methods

Cocrystals ratios	Method of cocrystal formation	Percent drug release	Increased percent drug release in folds
Lopinavir	-	41.15%	-
LOP: CIN (1:1)	Solvent evaporation method	79.78%	1.98
LOP: CIN (1:1)	Liquid assisted grinding	63.37%	1.53
LOP: CIN (1:1)	Ultrasonic assisted cocrystallization	72.41 %	1.71

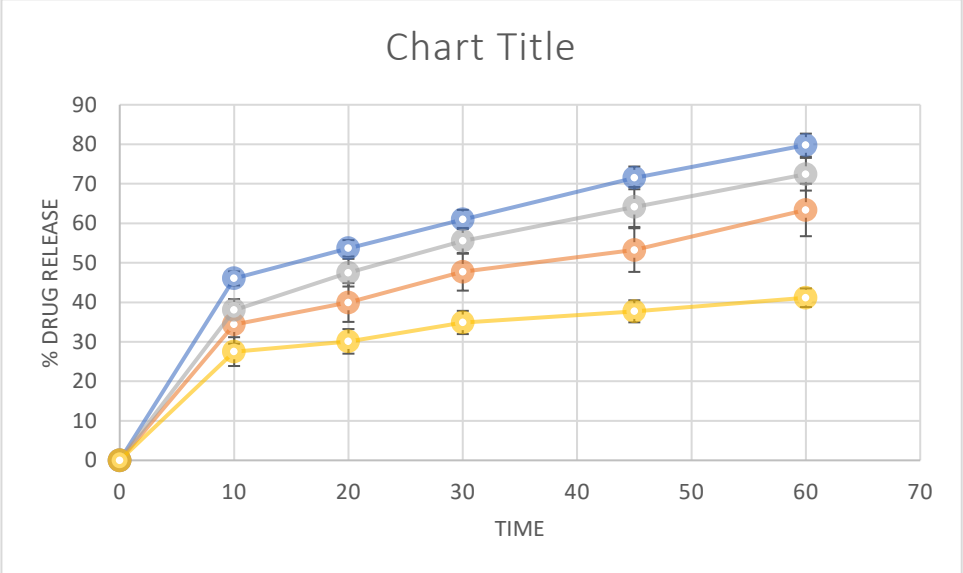

Fig. 3: Percent drug release for different methods

4. Evaluation for moisture uptake of lopinavir cinnamic acid cocrystals (n=3)

Lopinavir and Cocrystals (1:1) stoichiometric ratio, prepared by solvent evaporation, liquid assisted grinding and ultrasonic assisted cocrystallization gained 15% , 1.4%, 2.15%, and 0.8 % of weight respectively after 5 h

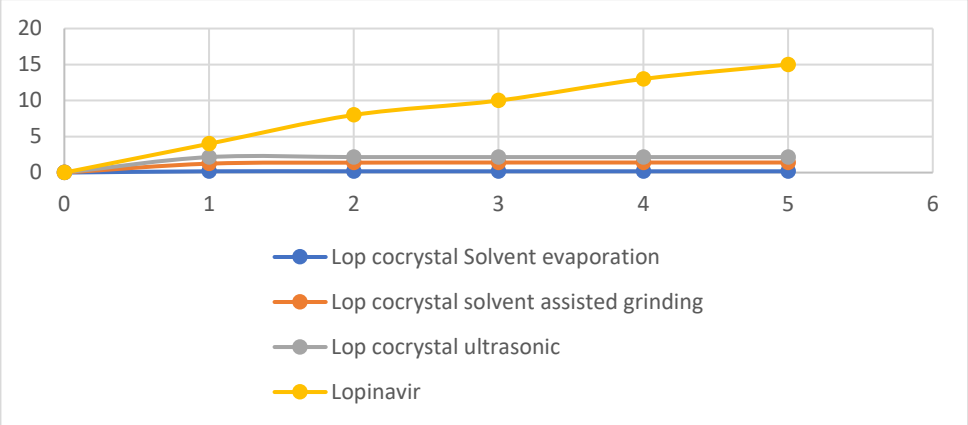

Figure 4 : moisture uptake of lopinavir cinnamic acid cocorystals

5.In vitro drug release

The in vitro dissolution profiles of the cocrystal were compared with that of lopinavir (figure 5). The in vitro dissolution rate of cocrystal was increased compared to the drug. Lopinavir shows 41.15% drug release after 60 min, whereas cocrystals show 79.78%. Dissolution rate was increased by 1.98 folds. The high dissolution rate of prepared cocrystal can be attributed to change in crystallinity of lopinavir due to possible hydrogen bond interaction with coformer. Mean N = 3.

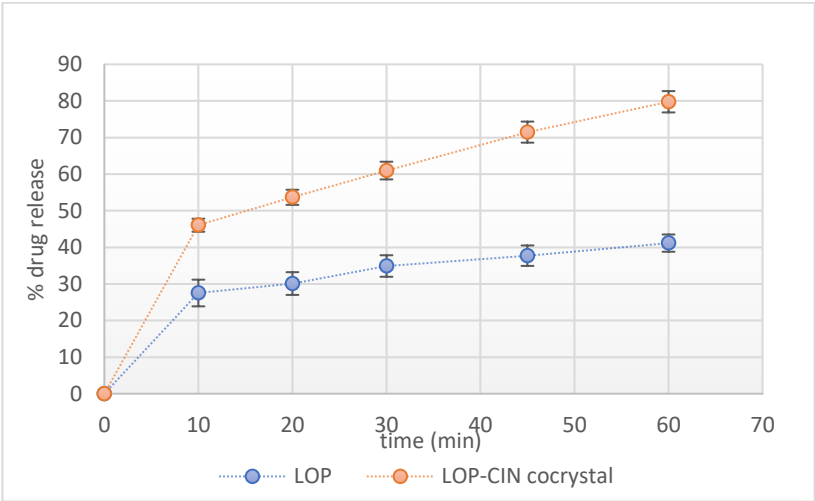

Fig. 5: In vitro drug release of lopinavir and Cocrystals

Cocrystals of a nonionizable drug can exhibit very different solubility-pH behaviors, depending on the coformer ionization properties. For a nonionizable and its 1:1 cocrystal (RHA) with an acidic coformer (HA) the cocrystal solubility is predicted to increase with pH and to decrease as the coformer solution concentration increases, coformer ionization properties and pKa values determine the solubility-pH dependence of cocrystals of a nonionizable drug.

6.Ex-vivo permeation study

The everted sac model is an efficient tool for studying in-vitro drug absorption mechanisms, intestinal metabolism of drugs, role of transporter in drug absorption, and for investigating the role of intestinal enzymes during drug transport through the intestine. The recommended tissue viability and metabolic activity of intestine under physiological conditions is approximately two hours. Appropriate care was taken to use the tissue within thisperiod of time.Lopinavir showed 41.72 % absorption. while lopinavir cinnamic acid cocrystal showed 65.14 % absorption fig 5. Increase in the absorption might be due to the increase in solubilityand dissolution rate. (n=3).

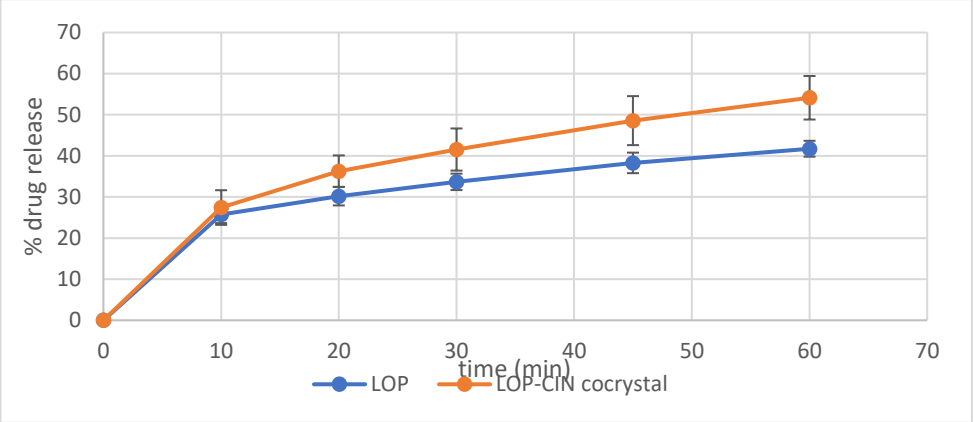

Fig. 6: Percentage drug absorption of lopinavir and Cocrystals

7.Micrometric flow properties of lopinavir-Cinnamic Acid cocrystal

Tablets were formulated by direct compression method and the co-crystals were evaluated for micrometric flow properties. Lopinavir-Cinnamic Acid co-crystal shows improved flow properties than lopinavir as shown in (Table 5).

Table 5: Micrometric flow properties of Lopinavir and Lopinavir-Cinnamic Acid co-crystal

Parameters	Lopinavir	Lopinavir: cinnamic acid (solvent evaporation)
Weight	5 gm	5 gm
Angle of repose	56.94^0 (very poor)	41.65^0 (passable)
Bulk density	0.166 gm/ml	0.312 gm/ml
Tapped density	0.25 gm/ml	0.41 gm/ml
Carr's index	33.3% (very poor)	25% (passable)
Hausner's ratio	1.5 (very poor)	1.33 (passable)

8.Experimental Design

The concentrations of lactose: MCC ratio and SSG were examined as two independent factors in the formulation of tablets using a 32 full factorial design. 9 experimental runs were used to determine the importance of individual and combined effects of lactose: MCC and SSG on % drug release.[Table: 6] The mean outcomes obtained by changing one element from its low to high value while maintaining another constant are known as the quantitative influence of independent variable in the resultant equation. Response surface methodology is a widely used method for formulating and optimising formulation variables. 3D response Surface Graphs were used to visualise the data.

Table 6. Experimental run & responses for optimization of co-crystal tablet using 32 full factorial design.

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Runs	Lactose: MCC	SSG	Hardness (Kg/cm2)	Friability (%)	% Drug release
1	80:20	20	4.2	0.89	60
2	90:10	12	2.95	0.95	45
3	80:20	12	2.8	0.84	44
4	70:30	20	3.2	0.83	58.5
5	70:30	12	2.6	0.81	45
6	90:10	16	4.5	0.96	49.07
7	80:20	16	2.4	0.86	43
8	90:10	20	4	0.95	59.23
9	70:30	16	3.5	0.82	48

9.Analysis of optimization data:

The formulations prepared as per the experimental design were evaluated and the analysis of experimental results were done by using Stat-Ease Design Expert. The ANOVA, P-value and Model F-value were obtained (Table 7).

The drug release between experimental batches varied between 43.0 % - 59.23%. The hardness varied between 2.9-4.2 Kg/cm 2, and friability between 0.81-0.96%. The response surface graph shows a direct dependence of hardness on lactose: MCC ratio, whereas SSG had a smaller lowering effect. The friability was improved slightly by both lactose MCC ratio and SSG. (Figure 5, 6,7).Thus, the formulation batch giving highest % drug release, lowest friability and highest hardness was chosen as the optimized batch based on desirability function (0.972). Thus, the optimized batch consisted concentration ratio of diluents that is lactose & MCC (80:20) and super disintegrant sodium starch glycolate.composition(16). The optimized formula was subjected to verification and no significant difference was found between the theoretical and the actual values of % drug release are given in Table 8.

%Friability = +0.88 + 0.067 * A +0.012 * B – 5.000E -003 * A* B
Hardness = +3.70 + 0.67 * A – 0.12 * B +0.000 *A*B
% drug release = +50.11 +0.33 *A + 7.17 * B + 0.25 *A*B

The interaction terms has very little lowering effect on all the three responses studies.
The two-factor interaction model was found to be significant with p values If the p-value is less than or equal to the significance level, you can conclude that there is a statistically significant association between the response variable and the term. Higher the value of R2 indicates better fit of model to data. Adequate precision denotes signal-to-noise ratio. It compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination.

Table 7: ANOVA output for optimization of Darunavir sorbitol co-crystal tablet

Sr no.	Outcomes	% Drug release	Friability	Hardness
1	Models	2FI	2FI	2FI
2	R2Value	0.8030	0.9418	0.8589
3	F – Value	6.80	26.95	10.14
4	P – Value	0.0325	0.0016	0.0145
5	Adequate Precision	5.779	12.72	7.819

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