

Enhanced Solubility and Dissolution of Drug-drug Cocrystals of Lopinavir-Ritonavir

Khushbu R Chaudhari^{1,*}, Jignasa K Savjani^{1,*}, Ketan T Savjani², Sandeep Dahiya², Jitendra O Bhangale³

¹Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, INDIA.

²Emcure Pharmaceuticals, Mehsana, Gujarat, INDIA.

³Department of Quality Assurance, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, INDIA.

ABSTRACT

Introduction: Drug-drug cocrystals of Lopinavir and Ritonavir were designed and characterized. In view of literature, Ritonavir increases the oral bioavailability of Lopinavir in low dosage. **Aim:** The present research aimed to improve the solubility, dissolution, and oral bioavailability of Lopinavir through a cocrystallization approach using Ritonavir as a cofomer. **Materials and Methods:** Cocrystallization was carried out using wet grinding and solvent evaporation methods. Prepared Cocrystals were examined and evaluated using characterization methods such as DSC, PXRD, FTIR, Polarized light microscopy, % Drug content, FE-SEM, Micromeritics properties, Solubility, Dissolution, and Stability studies as per ICH guidelines. **Results:** Developed cocrystals were showed superior solubility and dissolution as compared to pure drug. After 48 hr, aqueous solubility of Lopinavir and Ritonavir co-amorphous formulation (LRCWG) was increased 3.7-fold in the wet grinding method, while Lopinavir and Ritonavir Cocrystals Prepared by Solvent Evaporation Method (LRCSE) indicated 5.9-fold increase compared to pure drug. The co-amorphous formulation was developed using wet grinding showed 86% drug release at 60 min, and cocrystals synthesized through solvent evaporation method showed 94% of drug release. **Conclusion:** Cocrystals produced using solvent evaporation approach were stable and showed excellent physico-chemical characteristics. The study concluded that the cocrystallization approach would prove to be a successful method to improve physico-chemical and mechanical properties.

Keywords: Lopinavir, Ritonavir, Multi-Drug Cocrystals, Solubility, Dissolution.

Correspondence:

Dr. Jignasa K Savjani

Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University, Sarkhej-Gandhinagar Road, Ahmedabad-38248, Gujarat, INDIA.
Email id: jignasa.savjani@nirmauni.ac.in

Received: 09-11-2022;

Revised: 12-01-2023;

Accepted: 20-02-2023.

INTRODUCTION

Pharmaceutical cocrystal is the most novel approach for enhancing the physico-chemical and mechanical properties of active ingredients without disrupting the covalent bonds that are part of the active constituent's molecular structure.¹ Multi-Drug Cocrystals (MDCs) are "two or more dissociable active components present in one crystalline arrangement in a specific stoichiometric ratio, in which the components interact primarily via a combination of ionic and nonionic interactions, hydrogen bonding, and also partial proton transfer."² Nowadays, Pharmaceutical companies showed more interest in developing unit dosage forms, having two or more pharmaceutical molecules. The concept of synergistic effect is the foundation for the development of multidrug formulations in which multi drugs work together to enhance the oral bioavailability of the principal

drug by retarding the intestinal efflux mechanism.³ Lopinavir, an antiretroviral agent, belongs to the protease inhibitor class and prevents viral replication which improves the patient's life expectancy and quality of life.⁴ Prophylactically, Lopinavir is administered by Human Immunodeficiency Virus (HIV) infected pregnant women.⁵ Also, Lopinavir is used in the treatment of HIV infection with severe acute malnourished pediatric patients.⁶ Lopinavir is also used in the treatment of many fungal disorders by inhibiting cell development in fungi.⁷ Additionally, Lopinavir exhibited anti-parasitic action for a few parasites illnesses.⁸ Recent studies reported Lopinavir alone or in conjunction with Ritonavir was found to be effective in the treatment of COVID patients.⁹ The enzyme cytochrome P4503A4 extensively processes Lopinavir in the gastrointestinal region and liver, and P-glycoprotein efflux has a substantial impact on Lopinavir absorption which is the main cause of low oral bioavailability. Lopinavir is a BCS (Biopharmaceutical Classification System) class II agent and shows poor water solubility and slow dissolution.¹⁰

In addition, several studies reported that Ritonavir in combination with other anti-HIV drugs including Lopinavir acts as a pharmacokinetic booster and enhances the oral bioavailability of



DOI: 10.5530/ijper.57.2s.33

Copyright Information :

Copyright Author (s) 2023 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : EManuscript Tech. [www.emanuscrit.in]

Lopinavir by inhibiting CYP3A4 and P-glycoprotein. Ritonavir increased the absorption of Lopinavir from the intestinal lumen to the systemic circulation.¹¹ Nowadays, fixed-dose combinations of Lopinavir and Ritonavir are also commercially available. The main objective of this research is to develop drug-drug cocrystals of Lopinavir with Ritonavir using a cocrystallization approach via wet grinding and solvent evaporation methods to increase Lopinavir's solubility, dissolution which directly contribute to improve oral bioavailability.

MATERIALS AND METHODS

Materials

Ritonavir (HPLC purity 99%) and Lopinavir (99% HPLC purity) were obtained as free samples from Emcure Pharmaceuticals, Pvt. Ltd., Gandhinagar, Gujarat, India. All experiments were performed using HPLC grade water and organic solvents which were procured from Finar Pvt. Ltd., Ahmedabad, Gujarat, India.

Synthesis of Cocrystals

Wet grinding and solvent evaporation methods were used to synthesize Lopinavir-Ritonavir Cocrystals (LRC). Lopinavir (Figure 1a) and Ritonavir (Figure 1b) were weighed in a 1:1 stoichiometric ratio.

In Wet grinding method, weighted quantity of Lopinavir and Ritonavir were grinded for 15 min in the presence of few drops of methanol and water using a mortar and pestle. The Sample (LRCWG) was dried and kept in vacuum desiccator for 24 hr.¹² In solvent evaporation method, Lopinavir and Ritonavir were dissolved in 12 mL mixture of methanol and water (8:2), and then warmed at 50°C for 8 min to get clear solution. The solution (LRCSE) was filtered and dried for 24 h at room temperature in a vacuum desiccator. The dried samples (LRCWG and LRCSE) were triturated using mortar and pestle and passed through sieve (#60-size) to get uniform particle size.¹³

Melting point analysis

Melting point of all the samples were analyzed using Digital melting point apparatus (Labtronics Ltd., Mumbai).

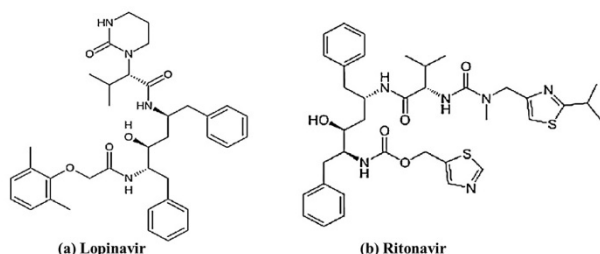


Figure 1: Molecular structure of (a) LPV and (b) RTV.

Differential Scanning Calorimetry (DSC)

Mettler Toledo instrument system was used to study the thermal analysis. The hermetically sealed standard pans held samples that ranged in weight from 5 to 10 mg at a heating rate of 10°C per minute (between 30°C and 350°C). Inert nitrogen was used to provide a controlled environment for the experiments, which were run at a flow rate of 40 mL/min.¹⁴

Powder X-ray Diffraction (PXRD)

X-ray source Cu-K radiation ($\lambda = 1.54060 \text{ \AA}$) was utilized in conjunction with the DIFFRAC.EVA V2. 1 software on a Bruker D8 advanced diffractometer to acquire PXRD. Samples were mounted on sample holders and scanned with steps of 0.01 ° (2 θ) and 0.1 sec per step, with a scanning interval range of 5-60 ° (2 θ).¹⁴

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR-spectra of all the samples were performed using FTIR-6100 Type A (JASCO Corporation, Tokyo, Japan) over a specific range of 4000-400 cm^{-1} using the KBr dispersion method.¹⁵

Polarized Light Microscopy

Each sample was spread on a clear glass slide and samples were observed under 10X magnification using polarized light microscope (Nikon, Kanagawa, Japan).

Field Emission-Scanning Electron Microscopy (FE-SEM)

The precise morphology of cocrystals was studied using FE-SEM equipment (ZEISS SIGMA HV, Germany) at a voltage of 15 kV. Palladium/gold layers 10 nm thick were sputtered on the samples after positioning. The material was then examined with magnifications ranging from 50X onwards.¹⁶

Drug content

Precisely weighed quantity of cocrystal equivalent to 10 mg of drug was dissolved in ethanol and volume was adjusted in 10 mL of volumetric flask. Absorbance was recorded using UV-spectrophotometer (Shimadzu UV1800). All the experiments were carried out in triplicate ($n=3$).¹⁷

Micromeritic characterization

Flow properties of powdered formulation have direct impact on particle size, shape, particle size distribution, surface texture, moisture content. Thus, variables that could provide insight into the flow of prepared cocrystals. Hence, Bulk density, Tapped density, Compressibility index, Hausner ratio, and Angle of repose were assessed.¹⁸

Bulk density (BD)

The BD was assessed by transferring the weighed quantity of sample into the 5mL of graduated cylinder. The mass to bulk volume ratio was recorded as Bulk density.

Tapped density (TD)

TD was examined by transferring the weighed quantity of sample into a 5 mL of measuring cylinder. The cylinder was placed in Tapped density apparatus (Electro lab). The cylinder having Initial volume (V_0) was recorded and subjected to tapping for 100 times then the final volume was recorded (V_t). The mass (M) to tapped volume ratio was recorded as TD. TD was calculated as: $TD = M / V_t$

Compressibility index (CI)

The CI is an indication of the compressibility of a powder. It was calculated by using formula: $CI = (TD - BD/TD) \times 100$

Hausner ratio (HR)

HR was determined using formula: $HR = TD/BD$

Angle of repose (AR)

This is used to measure the resistance to particle movement. AR was determined using equation: $AR (\theta) = \tan^{-1} (h/r)$

HPLC method for quantification

LPV was quantified using HPLC (Agilent Technology, Santa Clara, CA, USA) method which was available in the literature with required modifications. The mobile phase was 60:40 v/v of acetonitrile and potassium dihydrogen orthophosphate buffer pH 4 and chromatographic separation was achieved with Kromasil C_{18} (250 x 4.6mm, 5 μ m) column at ambient temperature. The flow rate was adjusted to 0.8 mL/min and the UV detection wavelength was 215 nm. Each sample with a 10 μ L volume was injected into the HPLC system.¹⁹

Saturation Solubility Study

Each sample in excess was added to 5 mL of double distilled water and vortexed initially for 5 min. The prepared suspensions were maintained in an orbital shaker for 48 hr at room temperature. Each sample was filtered using a 0.45 μ m sized membrane filter and measured by HPLC. All the experiments were carried out in triplicate ($n=3$).²⁰

Dissolution Study

All the samples were filled in empty size 0 capsules. In a temperature-controlled setting at $37 \pm 2^\circ\text{C}$, the dissolution study was carried out using the USP II (Paddle) method utilizing 900 mL of 0.1 N HCl as a dissolution media. The paddle stirring speed was maintained at 75 ± 2 rpm. 5mL of sample was withdrawn

from dissolution flask at 5, 10, 15, 20, 30, 45, and 60 min. The volume was kept constant by replacing the same amount with fresh dissolution media. Each experiment was carried out three times ($n=3$). Each sample aliquot was run through a 0.45 m nylon membrane filter, diluted as necessary, and then subjected to HPLC analysis.

The f_1 (the difference factor, between 0 and 15) and f_2 (the similarity factor, between 50 and 100) were calculated for both the formulation and compared with the marketed product using following formula.²¹

$$f_1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100$$

$$f_2 = 50 \cdot \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where, R_t is the % dissolved product for a reference batch at time point t, T_t is the % dissolved product for the test batch at time point t and n is the number of time points.

Stability study

For three months, a stability study of prepared samples were conducted at $40^\circ\text{C} / 75\% \text{ RH}$ and $25^\circ\text{C} / 60\% \text{ RH}$. The excess sample amount was stored in glass vials in a stability chamber. Solubility, % drug release, melting point, and % drug content were analyzed at 1, 2 and 3 months.

RESULTS

Melting point analysis

The prepared formulations LRCWG and LRCSE showed melting points in the range of 65°C - 67°C and 104°C - 106°C , respectively.

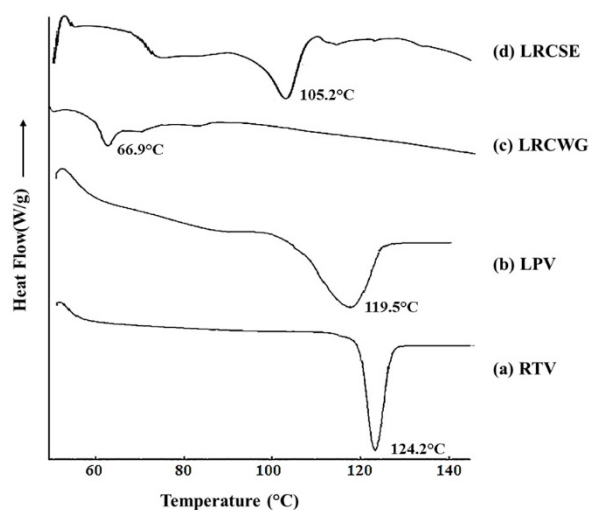


Figure 2: DSC thermograms of (a) RTV, (b) LPV, (c) LRCWG, and (d) LRCSE

Differential Scanning Calorimetry

The melting point of Ritonavir and Lopinavir were observed at 124.2°C and 119.5°C, respectively (Figure 2). These values matched with the earlier mentioned melting point values range in literature.^{22,23}

However, DSC scan of LRCWG showed a small peak at 66.9°C where nature should be confirmed with PXRD. The LRCSE showed a sharp endotherm at 105.2°C that might be the formation of new solid crystalline phase.²⁴

Powder X-ray Diffraction

The PXRD was performed to study possible molecular interactions.²⁴ The LPV diffractogram showed characteristic peaks at 2θ angles of 7.71, 8.51, 9.71, 10.51, 12.42, 14.72, 15.42, 16.42, 18.83, 21.63, and 22.64. The intense peaks in the diffractogram of crystalline RTV present at 2θ angles of 8.71, 8.81, 16.22, 20.13, and 21.83. These characteristic peaks were found to be disappeared in both formulation diffractograms which exhibited the formation of a new solid crystalline phase. The diffractogram of LRCWG exhibited the formation of a co-amorphous compound. The diffractograms of LRCSE, showed intense, sharp, and unique crystalline peaks, which were entirely different from the initial constituents (Figure 3). The LRCSE showed peaks at 6.70, 7.91, 8.71, 9.81, 10.61, 15.72, 17.32, 19.73, 21.63, 22.74, 24.74, and 26.44 2θ values revealing the development of a new phase of solid crystalline arrangement of cocrystals.²⁵

Fourier Transform Infrared Spectroscopy

The FTIR study was performed to observe hydrogen bonding and functional group interactions (Figure 4).

In LPV spectra, the peaks obtained at 3447 cm^{-1} and 3367 cm^{-1} indicated -OH and N-H stretching bands. The C-H frequency for aliphatic and aromatic carbon were observed from 2870 cm^{-1} -3060 cm^{-1} . The frequency of C=O of amide functional group was observed at 1701 cm^{-1} , 1655 cm^{-1} , and 1526 cm^{-1} . The C-N stretching vibration bands appeared at 1423 cm^{-1} .²² In RTV spectra, -OH stretching vibration band was observed at 3321 cm^{-1}

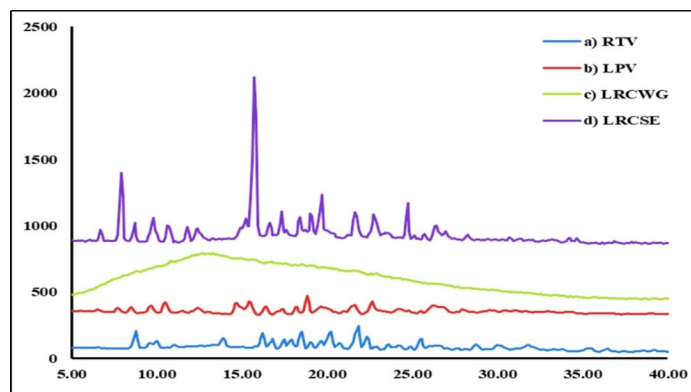


Figure 3: Powder X-ray diffractograms of (a) RTV, (b) LPV, (c) LRCWG, and (d) LRCSE.

and N-H stretching at 2962 cm^{-1} and 2851 cm^{-1} . Also, the C=O of amide functional group was observed at 1701 cm^{-1} , 1661 cm^{-1} . The Physical Mixture (LRPM) showed characteristic peaks of pure drugs. The peaks were observed for -OH group (3370 cm^{-1}), N-H stretching (3496 cm^{-1}), and C=O group of amides (1717 cm^{-1} and 1655 cm^{-1}).

The LRCWG showed peaks for -OH group (3383 cm^{-1}), N-H stretching (3406 cm^{-1}), and C=O of amide (1717 cm^{-1} , 1637 cm^{-1}). The LRCSE showed changes in -OH peak value at 3371 cm^{-1} , and N-H stretching at 3502 cm^{-1} . The C=O of amides appeared at 1661 cm^{-1} , and 1636 cm^{-1} . Peak broadening after 3000 cm^{-1} onwards indicated hydrogen bond formation that confirms the cocrystal formation.²⁶

Polarized Light Microscopy

The sample of LRCWG observed particles which were aggregated (Figure 5c) where, sample of LRCSE showed more prominent, robust, and rectangular shaped crystals (Figure 5d). Polarized light microscopy results confirmed with FE-SEM results.²⁶

Field Emission-Scanning Electron Microscopy

The cocrystals synthesized via the solvent evaporation method were more intact due to more substantial bond formation attained in the presence of plenty of solvents. However, the wet grinding method showed smaller irregular-sized particles, which may be due to the impaction mechanism involved in the process.²⁷ The sample of LRCWG showed aggregated particles (Figure 6c) where LRCSE showed rectangular flat crystals (Figure 6d).

Drug content

Drug content of LRCWG and LRCSE was exhibited $99.95 \pm 1.34\%$ and $99.91 \pm 0.65\%$, respectively.²⁸

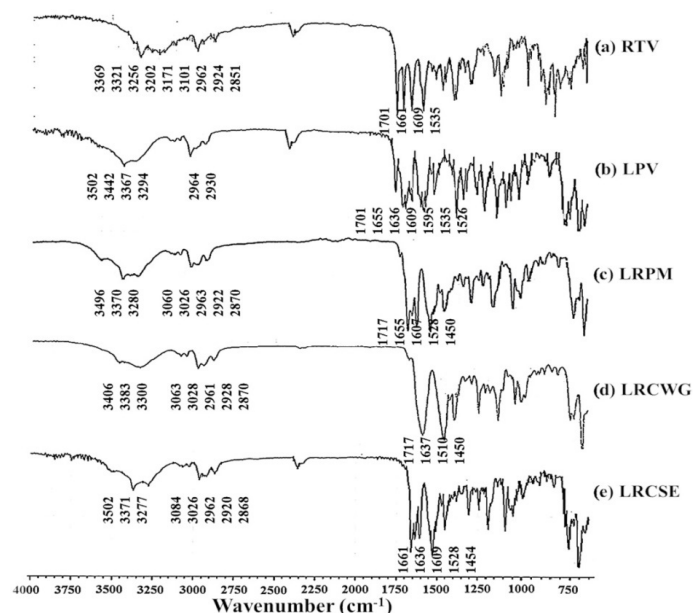
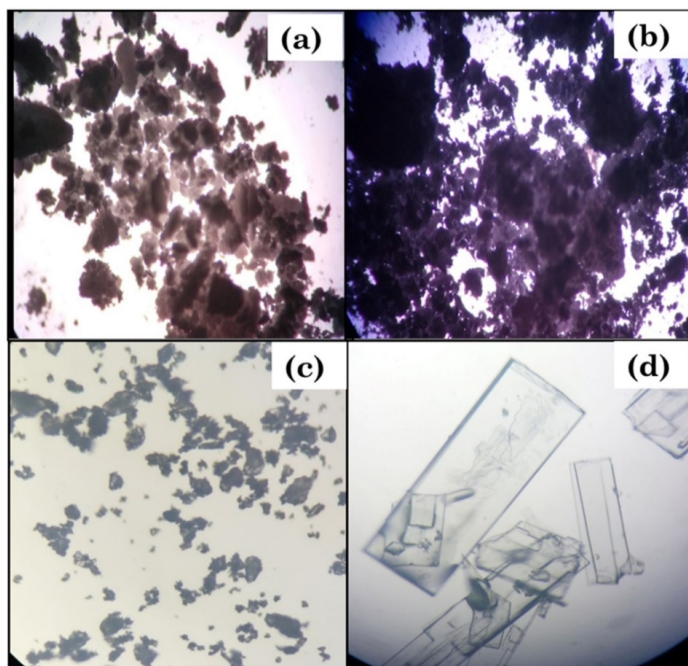


Figure 4: FTIR-spectra of (a) RTV, (b) LPV, (c) LRPM, (d) LRCWG, and (e) LRCSE.

Table 1: Micromeritic properties of LPV, RTV, LRCWG, and LRCSE.

Sample	Bulk density (g/mL)	Tapped density (g/mL)	Angle of repose (degree)	Compressibility index (%)	Hausner ratio
LPV	0.17 ± 0.21	0.29 ± 0.11	47.42 ± 0.57	36.79 ± 0.72	1.94 ± 0.42
RTV	0.13 ± 0.33	0.28 ± 0.17	56.32 ± 0.79	53.33 ± 0.64	2.14 ± 0.28
LRCWG	0.32 ± 0.24	0.43 ± 0.36	44.18 ± 0.82	28.72 ± 0.59	1.31 ± 0.62
LRCSE	0.45 ± 0.41	0.57 ± 0.16	27.31 ± 0.63	13.12 ± 0.71	1.08 ± 0.69

**Figure 5:** Polarized light microscopy of (a) RTV, (b) LPV, (c) LRCWG, and (d) LRCSE; 10X.

Micromeritic characterization

The flow properties (BD, TD, CI, HR, and AR) of prepared formulations showed better than pure drugs (Table 1). LRCWG showed CI=28.72 ± 0.59% and HR=1.31 ± 0.62 which indicated poor characteristics, while AR=44.18 ± 0.82 degrees showed passable characteristics. However, LRCSE showed good characteristics (CI=13.12 ± 0.71%), meanwhile LRCSE showed excellent characteristics (AR = 27.31 ± 0.63 degrees and HR=1.08 ± 0.69) as compared to pure drug and LRCWG.^{27,29}

Saturation Solubility Study

At room temperature, saturation solubility of LPV and RTV exhibited 6.13 ± 0.611 and 9.84 ± 1.614 µg/mL, respectively. However, LRCWG showed 22.44 ± 0.640 µg/mL and LRCSE showed 35.91 ± 2.244 µg/mL saturation solubility in water, which indicated 3.7-fold for LRCWG and 5.9-fold for LRCSE higher than LPV (Figure 7).³⁰

Dissolution Study

The dissolution study revealed % drug release of LPV as 29% at 60 min. However, LRCWG showed 86% and LRCSE showed 94% of

drug release at 60 min time intervals which enhanced dissolution profile compared to pure drug (Figure 8).

The order of % drug release at 60 min from higher to lower was observed as Marketed Product >LRCSE >LRCWG >RTV >LPV. The formulation prepared by solvent evaporation method showed higher % drug release than wet grinding method in 0.1N HCl. The F^1 and F^2 values of LRCWG and LRCSE were calculated (Table 2). As the f_1 value increases the test product and reference product dissimilarity in the dissolution profile increases. However, as the F^2 value is 100 the test and reference profiles are identical, and when approaches zero the dissimilarity increases.³¹

Stability study

Cocrystal's solubility and dissolution during storage could alter due to changes in crystallinity. Therefore, stable cocrystals with a faster rate of dissolution are preferred.

LRCSE was found to be stable in terms of % drug content, melting point, FTIR, solubility, and % drug release for three months at room temperature and accelerated conditions (Table 3). Also, FTIR revealed LRCWG was stable for one month in accelerated stability study while at room temperature, LRCWG was stable for two months. Moreover, LRCSE did not show any significant difference for three months (Figure 9).³²

DISCUSSION

Researchers have demonstrated cocrystallization has potential to alter physical characteristics of poorly soluble drug by modifying arrangement of a crystal lattice to improve solubility, dissolution and stability. The process consists of formation of a new solid crystalline phase from two different starting compounds.³³

Cocrystals were synthesized by wet grinding and solvent evaporation method. The wet grinding method, rearrangement occurs after breaking crystals in presence of few drugs of solvent resulted in formation of new phase with altered solid properties due to molecular interactions and hydrogen bonding. Also, solvent evaporation method was used to synthesize cocrystals. The dissolved starting components in solvent forms strong hydrogen bonds and molecular interactions and get sufficient time to strengthen the bonds.³⁴

The melting point of LRCWG and LRCSE was lowered as compared to pure drugs, which might be caused due to

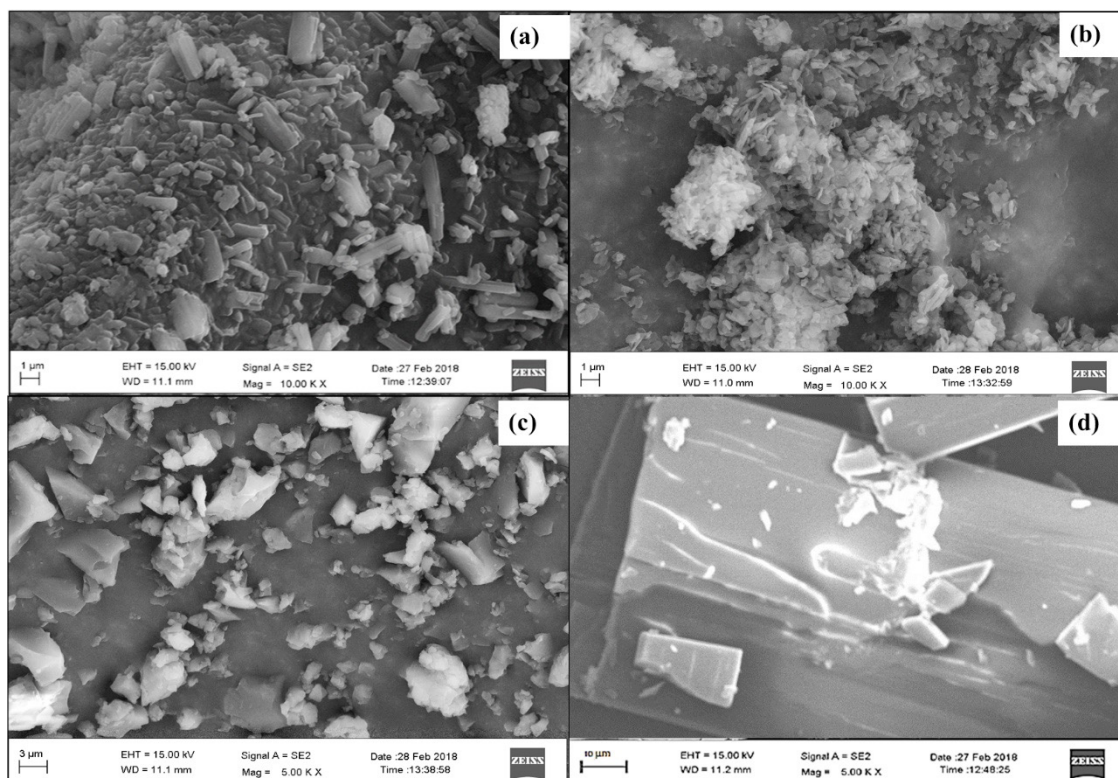


Figure 6: FE-SEM of (a) RTV, (b) LPV, (c) LRCWG, and (d) LRCSE.

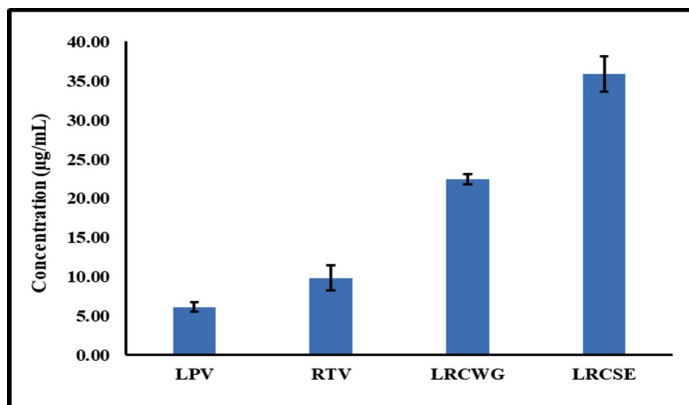


Figure 7: Solubility study of (a) LPV, (b) RTV, (c) LRCWG, and (d) LRCSE.

interactions between two initial components or by the emergence of a new solid phase with different physical characteristics.²⁷

The formation of new phases was obtained by DSC. However, the sharp LRCSE peak showed a highly crystalline nature of the cocryystals than the LRCWG peak. Further, The PXRD results also confirmed the highly crystalline nature of LRCSE and the co-amorphous form of the LRCWG solid compound.

In addition, the co-amorphous formulation prepared with the wet grinding method showed slightly less intense peaks compared to the cocryystals prepared with the solvent evaporation method, which might be due to the impactation mechanism involved during mechanical grinding that resulted in decrease in particle size and altered crystallinity.³⁵

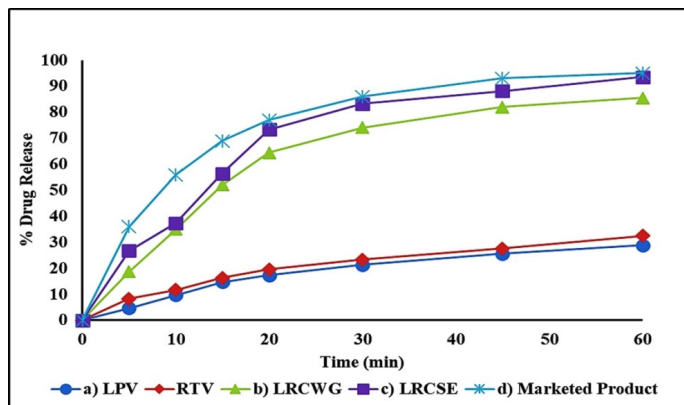


Figure 8: Dissolution profile of (a) LPV, (b) LRCWG, and (c) LRCSE, and (d) Marketed Product.

Table 2: f1 and f2 factor of LRCWG and LRCSE.

Formulation (Tt)	f1 value	f2 value
LRCWG	16.99	43.09
LRCSE	10.37	50.92

The FTIR-spectra of the cocryystal indicated molecular interactions and hydrogen bonding were present between individual components. FTIR confirmed that cocryystals were formed via the solvent evaporation method. Although LRCWG showed molecular interactions in FTIR, PXRD resulted in formation of a

Table 3: Stability study of LRCWG and LRCSE.

Parameters	Time points (months)	% Drug content	Melting point (°C)	FTIR	Solubility ($\mu\text{g/mL}$) after 48 hr	% Drug release at 1 hr
LRCWG 25°C \pm 60% RH	Initial	99.95 \pm 1.34	65-68	-	22.44 \pm 0.640	91
	1M	99.93 \pm 0.89	65-68	Not changed	22.58 \pm 0.891	93
	2M	96.17 \pm 1.78	65-68	Not changed	23.77 \pm 0.674	94
	3M	95.89 \pm 1.23	58-65	Changed	24.89 \pm 0.931	96
LRCWG 40°C \pm 75% RH	Initial	99.95 \pm 1.34	65-68	-	22.44 \pm 0.640	91
	1M	96.44 \pm 1.71	65-68	Not changed	23.73 \pm 0.621	94
	2M	94.89 \pm 0.56	58-65	Changed	25.67 \pm 0.533	95
	3M	96.45 \pm 0.82	58-65	Changed	26.61 \pm 0.279	97
LRCSE 25°C \pm 60% RH	Initial	99.91 \pm 0.65	102-107	-	35.91 \pm 2.244	94
	1M	99.56 \pm 1.12	102-107	Not changed	36.88 \pm 1.277	94
	2M	98.72 \pm 0.78	102-107	Not changed	36.69 \pm 2.354	93
	3M	96.43 \pm 0.91	102-107	Not changed	37.12 \pm 2.359	93
LRCSE 40°C \pm 75% RH	Initial	99.91 \pm 0.65	102-107	-	35.91 \pm 2.244	94
	1M	98.73 \pm 1.44	102-107	Not changed	38.11 \pm 2.645	94
	2M	97.22 \pm 0.45	102-107	Not changed	39.49 \pm 3.561	95
	3M	96.59 \pm 1.52	102-107	Not changed	40.34 \pm 2.816	95

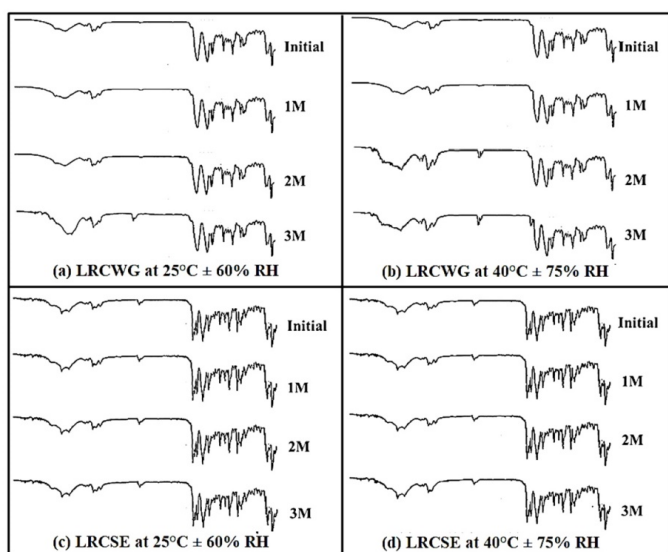


Figure 9: Stability Study indicating FTIR of (a) LRCWG at 25°C \pm 60% RH, (b) LRCWG at 40°C \pm 75% RH, (c) LRCSE at 25°C \pm 60% RH, and (d) LRCSE at 40°C \pm 75% RH

co-amorphous solid form. The FE-SEM and PLM also confirmed the formation of LRCSE cocrystals.

Crystal habit is very important criteria in cocrystallization process which have direct effect on mechanical properties, compressibility properties, and flow characteristics. Therefore, cocrystallization approaches and quantity of solvent in the process influence the crystal habit and thereby alter physico-chemical characteristics.¹⁶ The flow properties of LRCSE was improved in terms of BD, TD, AR, CI and HR. The f_1 factor of LRCSE was between 0 to 15

and F^2 values of LRCSE was between 50 to 100 which indicated similarity in reference (marketed product) and test (LRCSE) dissolution profile. However, f_1 and F^2 values of LRCWG showed slight dissimilarity in dissolution profile.³¹

The aqueous solubility of both formulations was improved. Similarly, the dissolution profile of LRCSE showed a higher % drug release at 60 min than LRCWG. There was no significant difference was observed in the dissolution pattern between both formulations.

It was noted that LRCSE in terms of % drug content, melting point, IR, solubility, and % drug release did not show significant differences during stability study. Therefore, LRCSE was stable for three months at room temperature and accelerated storage conditions.³⁶ The LRCSE was stable due to improved crystalline properties. On contrary, LRCWG stability was not stable for all three months at room temperature or accelerated stability conditions which might be due to amorphization during wet grinding method.

CONCLUSION

The solvent evaporation method successfully generated novel drug-drug cocrystals of the antiretroviral drugs Lopinavir and Ritonavir. However, the wet grinding method revealed the formation of a co-amorphous compound. When compared to pure drugs, the produced cocrystals performed better in terms of solubility, and dissolution.

To attain desired performance in the future, cocrystallization is a process used to transform poorly soluble pharmaceuticals into solid dosage formulations.

ACKNOWLEDGEMENT

The authors would like to thank Emcure Pharmaceuticals, Gandhinagar, Gujarat, India for supporting this research project.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

HIV is a chronic and complex disease in which treatment combines two or more drugs to target drug-resistant viruses. Highly active antiretroviral therapy (HAART) allows multiple-drug combination therapy to reduce the mortality rate. Also, to improve patient compliance, pharmaceutical companies are interested in developing unit dosage form consisting combination of two or more drugs. The cocrystallization approach has been successful in altering physico-chemical properties without changing the chemical formula of the compound. The cocrystallization strategy improves the physico-chemical and mechanical properties by forming hydrogen bonding and weak molecular interactions. Ritonavir in low doses is prescribed with many other antiretroviral agents to improve the oral bioavailability of the main drug. The cocrystallization approach is the best strategy to develop a unit dosage form of multiple drugs to improve the efficacy of the pharmaceutical product. This technique is not limited to the treatment of HIV but it has been useful for many other categories of drugs as well. Many issues like stability, moisture absorption, powder flow properties, and altering drug release can be solved through cocrystallization.

REFERENCES

- Schultheiss N, Newman A. Pharmaceutical cocrystals and their physicochemical properties. *Cryst Growth Des.* 2009;9(6):2950-67. doi:10.1021/cg900129f
- Basavoju S, Boström D, Velaga SP. Indomethacin-Saccharin Cocrystal: Design, Synthesis and Preliminary Pharmaceutical Characterization. *Pharm Res.* 2008;25(3):530-41. doi:10.1007/s11095-007-9394-1
- Trasi NS BSZQTL. Amorphous solid dispersion formation via solvent granulation—A case study with ritonavir and lopinavir. *Int J Pharm.* Published online December 1, 2019.
- Pham HT, Mesplede T. The latest evidence for possible HIV-1 curative strategies. *Drugs Context.* 2018;7:1-14. doi:10.7573/dic.212522
- Fraichard C, Bonnet-Serrano F, Laguillier-Morizot C, *et al.* Protease Inhibitor Anti-HIV, Lopinavir, Impairs Placental Endocrine Function. *Int J Mol Sci.* 2021;22(2):683. doi:10.3390/ijms22020683
- Owor M, Tierney C, Ziemba L, *et al.* Pharmacokinetics and Safety of Zidovudine, Lamivudine, and Lopinavir/Ritonavir in HIV-infected Children with Severe Acute Malnutrition in Sub-Saharan Africa: IMPAACT Protocol P1092. *Pediatric Infectious Disease Journal.* 2021;40(5):446-452. doi:10.1097/INF.0000000000003055
- Santos ALS, Braga-Silva LA, Gonçalves DS, *et al.* Repositioning Lopinavir, an HIV Protease Inhibitor, as a Promising Antifungal Drug: Lessons Learned from *Candida albicans*—*in silico*, *in vitro* and *in vivo* Approaches. *Journal of Fungi.* 2021;7(6):424. doi:10.3390/jof7060424
- Rebello KM, Andrade-Neto V V, Zuma AA, *et al.* Lopinavir, an HIV-1 peptidase inhibitor, induces alteration on the lipid metabolism of *Leishmania amazonensis* promastigotes. *Parasitology.* 2018;145(10):1304-1310. doi:10.1017/S0031182018000823
- Chouchana L, Boujaafar S, Gana I, *et al.* Plasma Concentrations and Safety of Lopinavir/Ritonavir in COVID-19 Patients. *Ther Drug Monit.* 2021;43(1):131-5. doi:10.1097/FTD.0000000000000838
- Donato EM, Martins LA, Fröhlich PE, Bergold AM. Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on *in vivo* data. *J Pharm Biomed Anal.* 2008;47(3):547-52. doi:10.1016/j.jpba.2008.02.014
- Rehman S, Nabi B, Fazil M, *et al.* Role of P-Glycoprotein Inhibitors in the Bioavailability Enhancement of Solid Dispersion of Darunavir. *Biomed Res Int.* 2017;2017:1-17. doi:10.1155/2017/8274927
- Chhajed SS, Rajderkar YR, Tajanpure AB, Sangshetti JN, Mahapatra DK, Kshirsagar SJ. Solvent Drop Grinding Approach Assisted Development of Glimpiride Co-crystals: Solubility Enhancement Journey of BCS Class-II Product. *Indian Journal of Pharmaceutical Education and Research.* 2020;54(3):602-9. doi:10.5530/ijper.54.3.109
- Budiman A, Megantara S, Saraswati P. Synthesize Glibenclamide-Ascorbic Acid Cocrystal using Solvent Evaporation Method to Increase Solubility and Dissolution Rate of Glibenclamide. *Res J Pharm Technol.* 2019;12(12):5805. doi:10.5958/0974-360X.2019.01005.9
- Patil S, Ujalambkar V, Mahadik A. Electrospray technology as a probe for cocrystal synthesis: Influence of solvent and coformer structure. *J Drug Deliv Sci Technol.* 2017;39:217-22. doi:10.1016/j.jddst.2017.04.001
- Sharma OP, Patel V, Mehta T. Design of experiment approach in development of febuxostat nanocrystal: Application of Soluplus® as stabilizer. *Powder Technol.* 2016;302:396-405. doi:10.1016/j.powtec.2016.09.004
- Deng JH, Lu TB, Sun CC, Chen JM. Dapagliflozin-citric acid cocrystal showing better solid state properties than dapagliflozin. *European Journal of Pharmaceutical Sciences.* 2017;104:255-261. doi:10.1016/j.ejps.2017.04.008
- E. R. Gaikwad SSKTBSSAP. Preparation and Characterization of Molecular Complexes of Fenofibrate Cocrystal. *Asian J Pharm.* 2017;11(4).
- Chinthaginjala H, Ahad HA, Bhargava E, Pradeepkumar B. Central Composite Design Aided Formulation Development and Optimization of Clarithromycin Extended-Release Tablets. *Indian Journal of Pharmaceutical Education and Research.* 2021;55(2):395-406. doi:10.5530/ijper.55.2.77
- Ravi PR, Vats R, Dalal V, Murthy AN. A hybrid design to optimize preparation of lopinavir loaded solid lipid nanoparticles and comparative pharmacokinetic evaluation with marketed lopinavir/ritonavir coformulation. *Journal of Pharmacy and Pharmacology.* 2014;66(7):912-26. doi:10.1111/jpph.12217
- Shete A, Murthy S, Korpale S, *et al.* Cocrystals of itraconazole with amino acids: Screening, synthesis, solid state characterization, *in vitro* drug release and antifungal activity. *J Drug Deliv Sci Technol.* 2015;28:46-55. doi:10.1016/j.jddst.2015.05.006
- Kassaye L, Genete G. Evaluation and comparison of *in-vitro* dissolution profiles for different brands of amoxicillin capsules. *Afr Health Sci.* 2013;13(2). doi:10.4314/ahs.v13i2.25
- Fayed ND, Arafa MF, Essa EA, El Maghraby GM. Lopinavir-menthol co-crystals for enhanced dissolution rate and intestinal absorption. *J Drug Deliv Sci Technol.* 2022;74. doi:10.1016/j.jddst.2022.103587
- Kogawa AC, Gutierrez Antonio S. Characterization of Darunavir: β -Cyclodextrin complex and Comparison with the Forms of Darunavir Ethanolate and Hydrate. *Journal of Pharmaceutical Sciences and Emerging Drugs.* 2016;04(01). doi:10.4172/2380-9477.1000110
- Hiendrawan S, Veriansyah B, Soewandhi SN, Wikarsa S. Simultaneous Cocrystallization and Micronization of Paracetamol-Dipicolinic Acid Cocrystal by Supercritical Antisolvent (SAS) Polyamines View Project Pharmaceutical Economics View Project. 2016. <https://www.researchgate.net/publication/287640829>
- Seo JW, Hwang KM, Lee SH, Kim DW, Park ES. Preparation and characterization of adefovir dipivoxil-stearic acid cocrystal with enhanced physicochemical properties. *Pharm Dev Technol.* 2018;23(9):890-9. doi:10.1080/10837450.2017.1334664
- Chaudhari KR, Savjani JK, Savjani KT, Shah H. Improved pharmaceutical properties of ritonavir through co-crystallization approach with liquid-assisted grinding method. *Drug Dev Ind Pharm.* 2022;1-10. doi:10.1080/03639045.2022.2042553
- Panzade P, Shendarkar G. Design and preparation of zaltoprofen-nicotinamide pharmaceutical cocrystals via liquid assisted grinding method. *Indian Journal of Pharmaceutical Education and Research.* 2019;53(4):S563-70. doi:10.5530/ijper.53.4.s.151
- Laxmi P, Varma A, Pai A, Sathyannarayana MB. Experimental Data of Fabricated Co-crystals of Doxorubicin HCl with Flavonoids. *Indian Journal of Pharmaceutical Education and Research.* 2019;53(3s):s225-30. doi:10.5530/ijper.53.3s.91
- Panzade P, Shendarkar G, Shaikh S, Balmukund Rathi P. Pharmaceutical Cocrystal of Piroxicam: Design, Formulation and Evaluation. *Adv Pharm Bull.* 2017;7(3):399-408. doi:10.15171/apb.2017.048

30. Patil S, Chaudhari K, Kamble R. Electrospray technique for cocrystallization of phytomolecules. *J King Saud Univ Sci.* 2018;30(1):138-41. doi:10.1016/j.jksus.2017.04.001
31. Sokal A, Pindelska E, Szeleszczuk L, Kolodziejcki W. Pharmaceutical properties of two ethenzamide-gentic acid cocrystal polymorphs: Drug release profiles, spectroscopic studies and theoretical calculations. *Int J Pharm.* 2017;522(1-2):80-9. doi:10.1016/j.ijpharm.2017.03.004
32. Hiendrawan S, Veriansyah B, Widjojokusumo E, Soewandhi SN, Wikarsa S, Tjandrawinata RR. Physicochemical and mechanical properties of paracetamol cocrystal with 5-nitroisophthalic acid. *Int J Pharm.* 2016;497(1-2):106-13. doi:10.1016/j.ijpharm.2015.12.001
33. Blagden N, De Matas M, Gavan PT, York P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. *Adv Drug Deliv Rev.* 2007;59(7):617-30. doi:10.1016/j.addr.2007.05.011
34. Douroumis D, Ross SA, Nokhodchi A. Advanced methodologies for cocrystal synthesis. *Adv Drug Deliv Rev.* 2017;117:178-95. doi:10.1016/j.addr.2017.07.008
35. Emami S, Siahi-Shadbad M, Barzegar-Jalali M, Adibkia K. Feasibility of electrospray deposition for rapid screening of the cocrystal formation and single step, continuous production of pharmaceutical nanococrystals. *Drug Dev Ind Pharm.* 2018;44(6):1034-47. doi:10.1080/03639045.2018.1430821
36. Goud NR, Suresh K, Sanphui P, Nangia A. Fast dissolving eutectic compositions of curcumin. *Int J Pharm.* 2012;439(1-2):63-72. doi:10.1016/j.ijpharm.2012.09.045

Cite this article: Chaudhari KR, Savjani JK, Savjani KT, Dahiya S, Bhangale JO. Enhanced Solubility and Dissolution of Drug-drug Cocrystals of Lopinavir-Ritonavir. *Indian J of Pharmaceutical Education and Research.* 2023;57(2s):s292-s300.