



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203





Human Journals

Research Article

August 2020 Vol.:19, Issue:1

© All rights are reserved by ASHISH V. THER et al.

Pharmaceutical Cocrystal of Cilnidipine- Benzoic Acid (Design, Formulation, and Evaluation)

 <p>IJPPR INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH An official Publication of Human Journals</p> 
<p>¹ASHISH V. THER, ²YOGESHWARY M. BHONGADE, ³ANAND D. SAWKARE</p> <p>¹ Student of Pharmacy, Department of Pharmaceutics, ² Student of Pharmacy, Department of Pharmacology,</p> <p>³Assistant Professor, Department of Pharmaceutics. Maratha Vidya Prasarak Samaj College of Pharmacy, Nashik, India</p> <p>Submission: 24 July 2020 Accepted: 30 July 2020 Published: 30 August 2020</p>

Keywords: Cilnidipine, Co-crystals, CCF's, DSC, PXRD

ABSTRACT

The objective of the present study was to prepare and evaluate pharmaceutical co-crystals of Cilnidipine using different co-crystal formers. Co-crystals were prepared by solid-state grinding and solvent evaporation method using different API: CCF ratios. Organic acids like Benzoic acid, Citric acid, Boric acid, and saccharin sodium were used as cocrystal former. Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD) studies support the formation of cocrystals with benzoic acid as CCF by both the methods. A comparison pre-formulation characterization and dissolution rate of co-crystal with pure API shows improvement in both. Prepared co-crystal shows improvement in aqueous solubility than the parent drug.



HUMAN JOURNALS

www.ijppr.humanjournals.com

INTRODUCTION

An Active Pharmaceutical Ingredient (API) can present in different solid-state forms, which include polymorphs, solvates, salts, co-crystals, and amorphous forms.¹ Each form exhibits distinctive physicochemical properties that can immensely influence the bioavailability, stability, manufacturability and other performance characteristics of the formulated API.² Such multiplicity offers the opportunity of fine-tuning key physicochemical properties of the pharmaceutical product without compromising the pharmacological activity of the API as the molecular structure is conserved.³

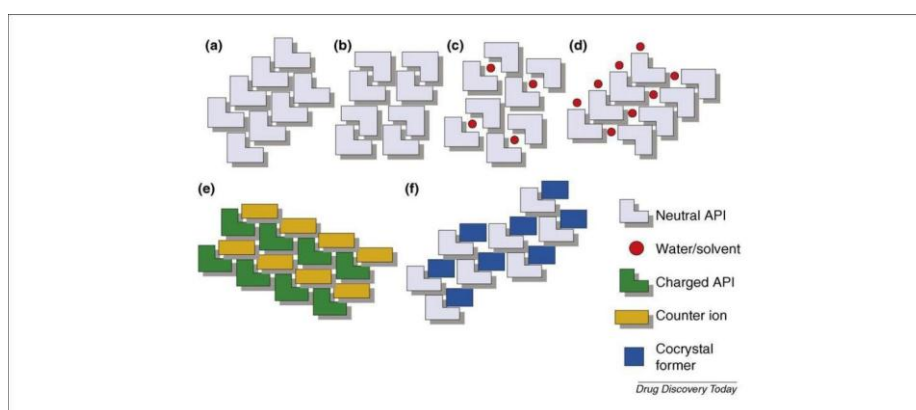


Figure No. 1: The range of single-crystalline forms that are possible for an API: (a) pure API; (b) polymorph of pure API; (c) clathrate hydrate/solvate of API; (d) hydrate/solvate of API; (e) salt of API; (f) pharmaceutical co-crystal. Salts and co-crystals can also form hydrates, solvates, and polymorphs.

PHARMACEUTICAL CO-CRYSTALS

Co-crystals, a class of composite for which the principles of crystal engineering are employed, have gained a lot of recent awareness owing to their agreeability to design and their ability to customize physicochemical properties. They represent a class of compounds with a large prospective and play a prime part in chemical and pharmaceuticals chiefly in the field of polymorphism.

Pharmaceutical co-crystals encompass of API and pharmaceutically accepted compounds. Cocrystals are formed because of various types of interactions containing hydrogen bonding, Van-der Waal's forces, and π - π stacking, and conducive geometries during self-assembly are responsible for the creation of supra-molecular webbing. Co-crystals are several component

systems whose components link by hydrogen bonding or other weak intermolecular interactions somewhat by an ion-pairing. A detailed understanding of the supramolecular chemistry of the functional groups present in the given molecule is the first step in designing a co-crystal since it makes an easy selection of molecules that contain the suitable appreciative functional groups. Here these appreciative functional groups are referred to as co-crystal formers. ⁴

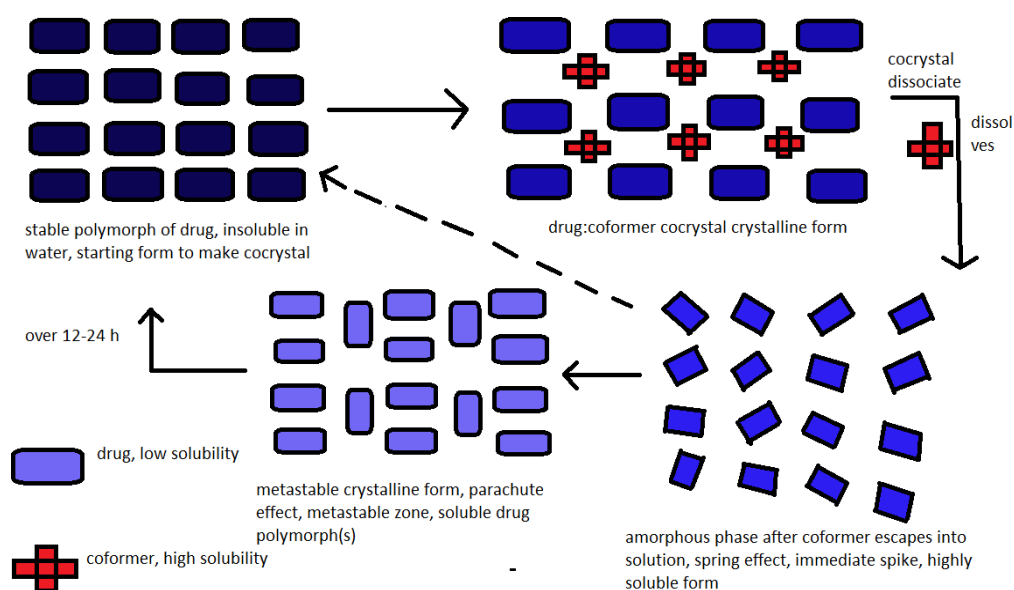


Figure No. 2: Mechanism for solubility advantage of pharmaceutical co-crystals.

PROPOSED MECHANISM FOR THE SOLUBILITY ADVANTAGE OF PHARMACEUTICAL CO-CRYSTAL ^(5, 6)

The disconnection of the hydrogen-bonded co-crystal in the aqueous medium delivers the more soluble co-former into the solution, whereas the less soluble drug molecules mass as an amorphous phase because of the unanticipated hit from solution. These mass lack the long-range order and cyclical characteristics of the crystalline state. The amorphous phase gives peak drug solubility for a tiny period (the spring), which will moderately transform to metastable polymorph(s) and thereby expand the metastable zone width (the parachute effect). Finally, the drug will transfigure to the stable, insoluble polymorph, but by this time the bulk of the drug has been absorbed through the fast-dissolving metastable state(s). The Ostwald's Law of Stages could stretch the metastable zone width to sundry hours. If the amorphous state directly transforms into the stable, crystalline form without the intermediacy of metastable polymorphs (dash arrow), the drug will show spring effect.

MATERIAL AND METHOD

PREPARATION OF PHARMACEUTICAL CO-CRYSTALS^(1, 6- 11)

The Cilnidipine– Benzoic Acid Co-Crystal

The solvent evaporation method was employed for the preparation of Cilnidipine– Benzoic acid co-crystal. Equimolar quantities of Cilnidipine (492.52 mg, 1 mmol) and Benzoic acid (122.12 mg, 1 mmol) were mixed with molar ratio (1:1). Cilnidipine– Benzoic acid (1:1) was dissolved in 10 ml of methanol and allowed to evaporate slowly at room temperature.

CHARACTERIZATION OF CO-CRYSTALS^(8, 11, 12)

MELTING POINT⁽¹³⁾

The melting point was taken by a simple capillary method using melting point apparatus. The melting point of API and CCF's were noted. Each observation was made in triplicate.

SATURATION SOLUBILITY DETERMINATION OF REPARED CO-CRYSTALS⁽¹⁴⁾

Saturation solubility study was conducted in different media (water). Each batch of prepared co-crystals in excess amount was added to 10 ml of each media in a 10 ml volumetric flask and then kept for sonication (CITIZEN LAB CD 4820). After the equilibration period of 24hr, the samples were removed. The supernatant was then analyzed by a UV-visible spectrophotometer (SHIMADZU 2450) at a respective wavelength with appropriate dilutions with respected media.

FT-IR SPECTROSCOPY

FT-IR of all samples was performed on the FTIR instrument (SHIMADZU 2450S). FT-IR of co-crystals was taken by preparing the KBr pellet. The spectra were collected over the range of 4000-500 cm^{-1} .

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Thermal analysis by DSC of Co-crystals was performed using Differential Scanning Calorimetry DSC- Mettler 1 star calorimeter. Sample powders were placed in aluminum pans,

sealed, and were heated from 50°C to 250°C under constant purging dry nitrogen flow. An empty aluminum pan was used as a reference.

POWDER X-RAY DIFFRACTION (PXRD)

X-ray diffraction is by far the most popular method used to characterize crystalline materials. PXRD allows conventional ‘fingerprinting’ to identify the new co-crystal form(s) by comparing it with API and co-former.

PREFORMULATION CHARACTERIZATION OF SELECTED CO-CRYSTALS⁽¹⁵⁾

After the co-crystallization, we performed the Pre-formulation characterization of co-crystals and their comparison with Cilnidipine. The pharmaceutical processing properties i.e. angle of repose, bulk density, tapped density, Carr’s index. Hausner’s ratios were studied in comparison with Cilnidipine.

BULK DENSITY

Bulk density is defined as the ratio of the mass of powder to bulk volume. Method:

Weigh 2 gm. of Co-crystals sifted through 60 no sieve and transfer in 10 ml cylinder, carefully level the powder without compacting, and read apparent volume (V_0). Calculate bulk density as follows,

$$\text{Bulk Density} = \text{Weight of powder/Bulk volume}$$

TAPPED DENSITY

Weigh 2 gm. of Co-crystals sifted through 60 no sieve and transfer in 10 ml cylinder, mechanically tap the cylinder 100 times, and read tapped volume. Calculate tapped density as follows,

$$\text{Tapped Density} = \text{Weight of powder/Tapped volume}$$

COMPRESSIBILITY INDEX

The compressibility index measures the propensity of powder to be compressed. The packing ability of drugs was evaluated from a change in volume, which is due to the rearrangement of packing occurring during tapping. It is represented as Carr’s Compressibility index.

$$\text{Carr's Index (\%)} = [(TD-BD) \times 100]/TD$$

HAUSNER RATIO

It gives an idea regarding the flow of blend. It is the ratio of tapped density to bulk density. It is calculated as follows:

$$\text{Hausner Ratio} = TD/BD$$

ANGLE OF REPOSE

The angle of Repose is defined as the maximum angle that can be obtained between freestanding surfaces of a powder heap to the horizontal plane.

$$\theta = \tan^{-1} h/ r$$

Where: h=height of pile

r = radius of the base of pile θ = angle of repose

METHOD:

A funnel was held with a clamp such that the stem of the funnel is 2.5 cm above the graph paper is placed on horizontal surface. Weighed amount of powder was taken and poured into the funnel keeping the orifice blocked. The powder was allowed to flow by removing the blockage until the apex of the conical pile just touches the tip of the funnel. Height of pile and average of 3 diameters formed by a pile of powder was measured with the help of ruler and angle of repose was determined.

DRUG CONTENT

The amount of cilnidipine present in co-crystals determine by dissolving co-crystals equivalent to 10mg of cilnidipine into 100 ml of PBS pH 6.8 buffer and UV absorbance was measured at 224nm.

INTRINSIC DISSOLUTION RATE DETERMINATION ^(14, 15)

In vitro dissolution test was carried out using a USP type 2 apparatus. The dissolution medium chosen was PBS pH 6.8 (volume 900ml, temperature 37°C, speed 50 rpm). A 5 ml sample at

each sampling interval was withdrawn. After each sampling sample was withdrawn was replaced by the same dissolution media kept in the control flask. The sample withdrawn was immediately filtered and analyzed for sample content by UV spectrophotometer at 224nm after suitable dilution.

RESULTS AND DISCUSSION

SELECTION OF DRUG

The model drug of choice for co-crystallization should possess poor aqueous solubility. Other properties can be considered are as follows-

1. Poor compressibility
2. Poor flow property
3. As per the hydrogen bonding rule for co-crystal design, hydrogen acceptor and donor are available in this drug, so it is one of the criteria to select as a model drug.
4. As per pKa concept Δ pKa value of drug found less than one, thus it is selected as a model drug.

SELECTION OF CCFs ⁽¹⁶⁻¹⁷⁾

For current study Benzoic acid, Citric acid, Tartaric acid, Boric acid, Saccharin were used as co-crystal former.

SOLVENT SELECTION

The solvent selection was done based on mutual solubility is that the solubility parameter of the drug and that of CCF in common, do not differ too much. This requirement however is not sufficient hydrogen bonding provided by the solvent is also taken into consideration. After contemplating all criteria, methanol was selected as a solvent of choice.

CHARACTERIZATION OF DRUG & CCF's

MELTING POINT DATA OF DRUG AND CCF'S

The melting point was determined using a simple capillary method. Readings were taken in triplicate and the average was taken. The melting point of Cilnidipine was found in the range of 105-110°C.

And the melting point of the co-crystal former was found Benzoic acid (122°C), Citric acid (153°C), Saccharin (228°C), Boric acid (170°C), Tartaric acid (172 °C).

FTIR

FTIR DATA FOR DRUG

Major functional groups present in Cilnidipine show characteristic peaks in the FTIR spectrum. These peaks were observed at different wavenumbers and the functional group associated with these peaks. The major peaks were identical to the functional group of Cilnidipine. Hence, the purity of the sample was confirmed.

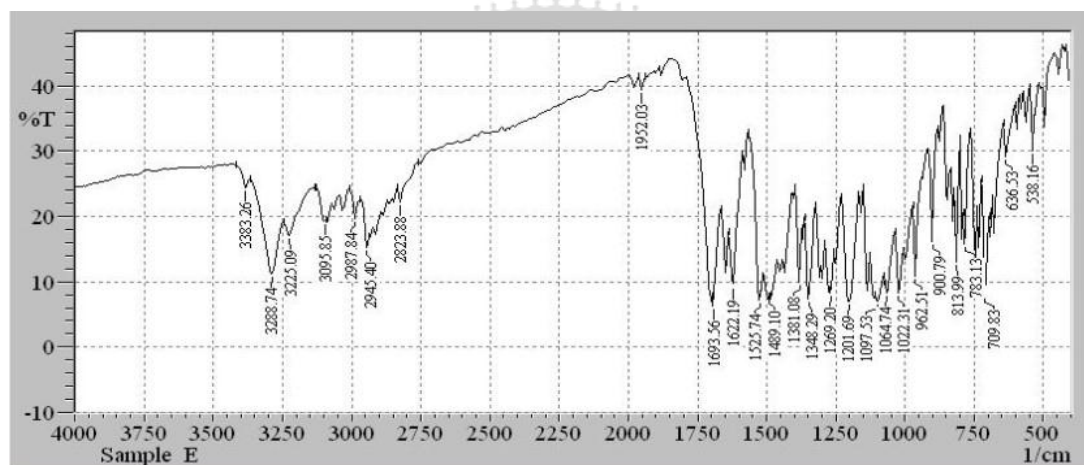


Figure No. 3: FT-IR spectrum of Cilnidipine

Table No. 1: Interpretation of the FT-IR spectrum of Cilnidipine

Functional group	Observed range	Standard range
NO ₂ (Nitro)	1348.29	1350-1550
C-H (Alkane) Stretching	2945.40	2850-3000
C-O (ether/ester)	1201.69	1000-1300
C-H (Aromatic) Stretching	3095.85	3050-3150
C=C (Aromatic) Stretching	1489.10	1475-1600
N-H(sec. amine) Stretching	3288.74	3100-3500

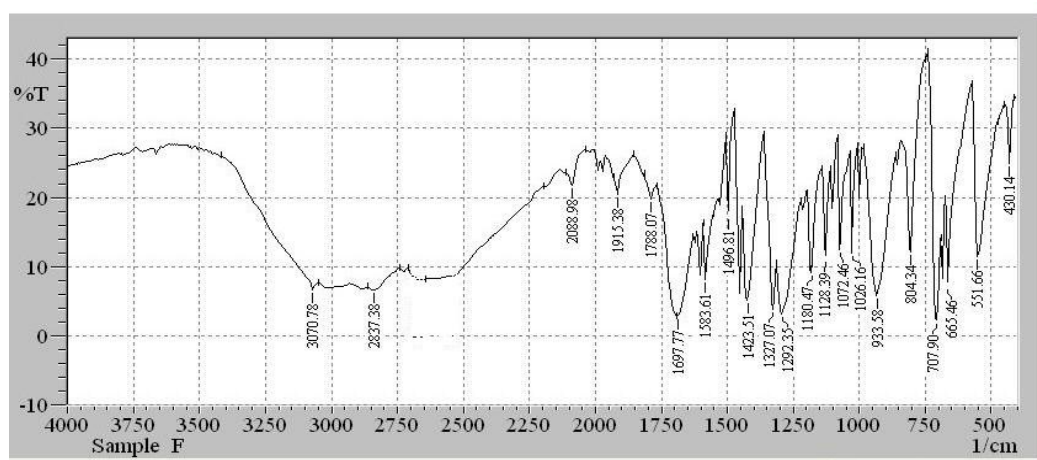


Figure No. 4: FTIR data for benzoic acid

FTIR DATA FOR BENZOIC ACID

Table No. 2: Interpretation of the FT-IR spectrum of Benzoic Acid

Functional group	Observed range	Standard range
C=O (Carboxylic acid)	1697.77	1700-1725
O-H (Carboxylic acid)	2837.38	2400-3400
C-H (Aromatic) Stretching	3070.78	3050-3150

DIFFERENTIAL SCANNING COLORIMETRY (DSC)

DSC DATA FOR DRUG

DSC of pure Cilnidipine showed a characteristic, sharp endothermic peak at 108.29°C, which is associate with the melting point of the drug & indicates the purity of Cilnidipine.

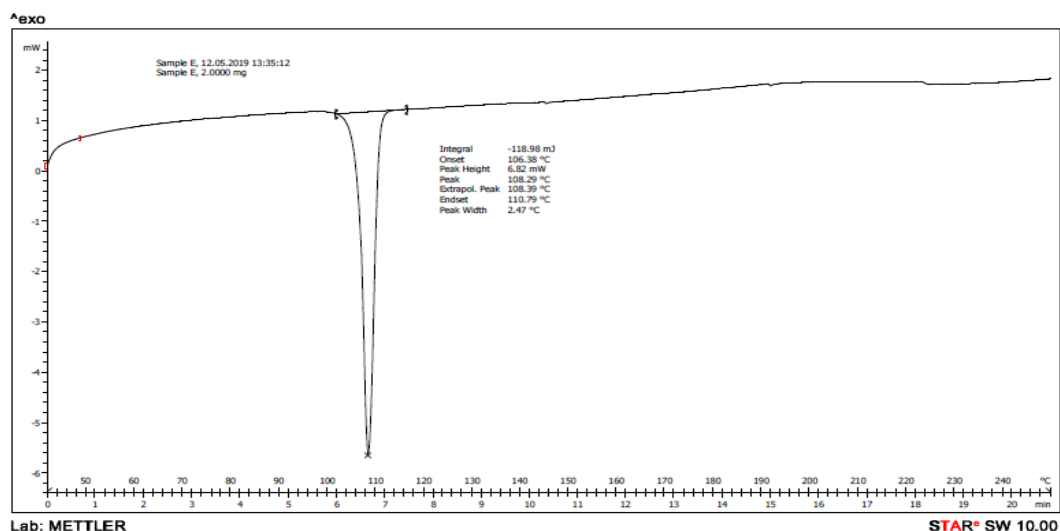


Figure No. 5: DSC thermogram of Cilnidipine

DSC data for Benzoic Acid

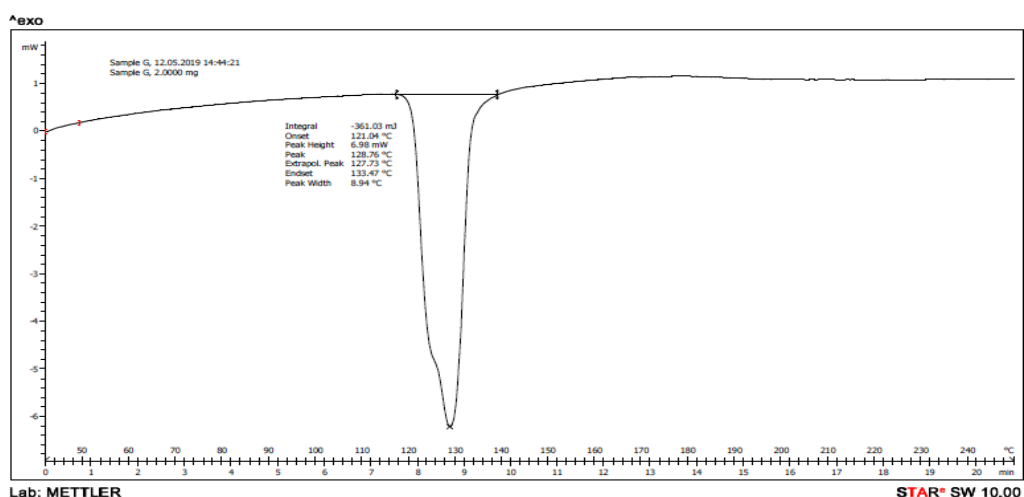


Figure No. 6: DSC thermogram of Benzoic Acid

CHARACTERISATION OF PREPARED CO-CRYSTALS

MELTING POINT

During melting point determination of co-crystal (cilnidipine- benzoic acid 1:1). Significant thermal change in co-crystal was observed 74-76°C which indicates the formation of co-crystal and this was further verified by DSC.

SATURATION SOLUBILITY DETERMINATION OF PREPARED CO-CRYSTALS

Citation: ASHISH V. THER et al. *Ijppr.Human*, 2020; Vol. 19 (1): 739-754.

Saturation solubility of co-crystal of cilnidipine with benzoic acid in a 1:1 ratio carried out in the water as a solvent to predict improvement of poor aqueous solubility of cilnidipine in water. Saturation solubility was found to be 3.146 mg/ml which was threefold of cilnidipine.

FOURIER- TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The N-H peak in the region 3288.74 cm^{-1} , nitro (N=O) peak of at the frequency 1348.29, and 1201.69 cm^{-1} for the C-O group of Cilnidipine was retained in all Co-crystal spectrum. In the Cocrystals of Cilnidipine with Benzoic acid as co-former by solution crystallization (sample B), distinctive peaks for hydrogen bonding conformation were observed in FT- IR spectrum. These characteristics peaks are as follows,

Hydrogen bonding in Co-crystals by FT-IR spectroscopy is detected by observing a decrease in intensity of -H peak and appearance of low frequency broad O-H band. Broad features were observed in the region of 2847.03 to 2567.34 cm^{-1} , indicating the occurrence of hydrogen bonding.

A decrease in O-H stretching frequency indicates that the hydroxyl group is participating in hydrogen bonding. The extent of hydrogen bonding can be determined in frequency and relative band broadening.

The lowering of frequency is the function of the degree and strength of hydrogen bonding.

Because of hydrogen bonding, the carbonyl stretching shifted towards the lower frequency (1697.77 cm^{-1} to 1687.41 cm^{-1}) indicated the presence of un-ionized carboxylic acid group (often $10\text{-}20\text{ cm}^{-1}$ decrease in frequency seen).

The significant changes observed in the region of the N-H and C=O stretching regions indicated the formation of a new hydrogen bond.

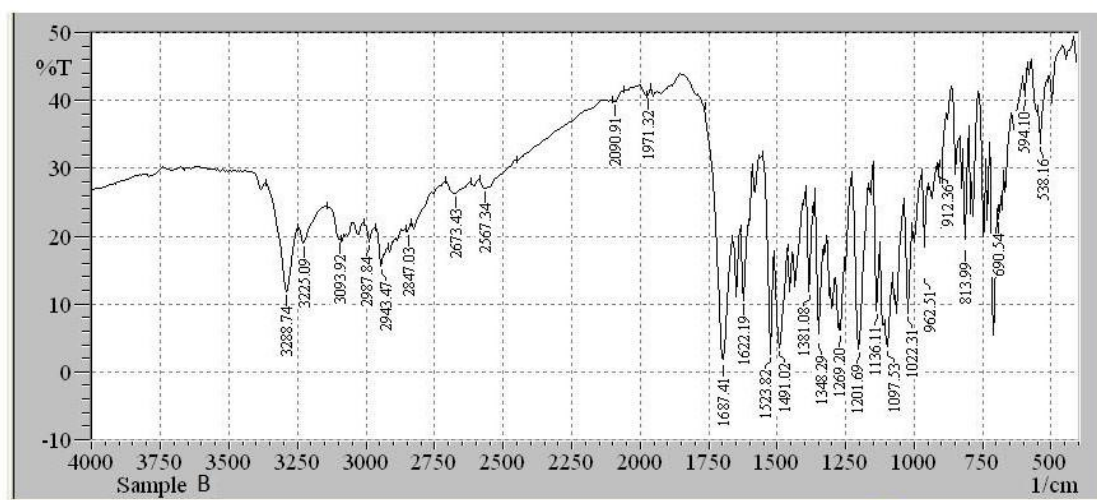


Figure No. 7: FTIR spectrum of co-crystal of CLD-Benzoic acid

SOLUBILITY STUDY OF API IN DIFFERENT MEDIA

Solubility of API in water (0.00638 mg/ml), Methanol (0.0228 mg/ml), PBS pH 6.8 (0.00379 mg/ml) were found.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

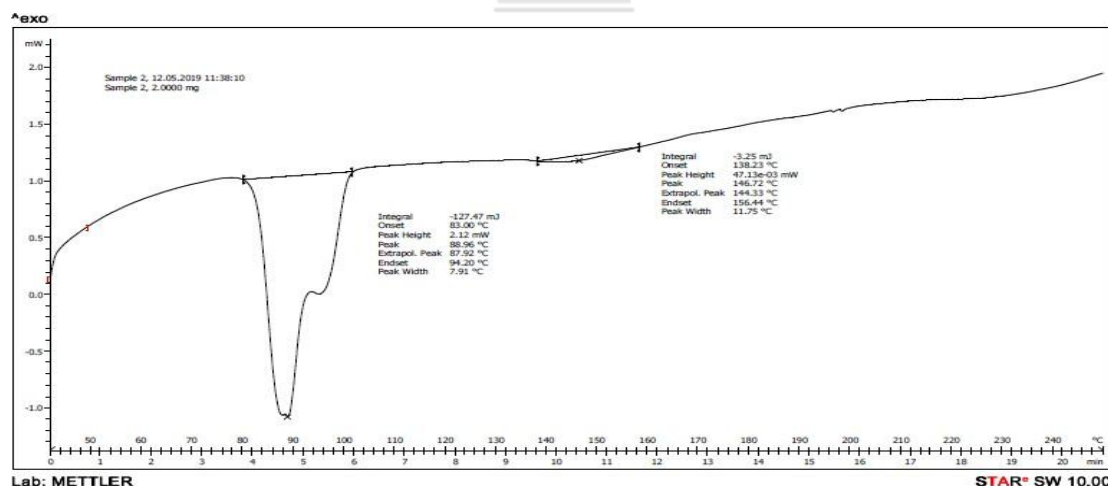


Figure No. 8: DSC thermogram of co-crystal of cilnidipine-Benzoic acid

From the result of the DSC study, Co-crystal formation can be confirmed for those formulations which show a characteristic change in the melting behavior. Such peculiar change in melting behavior confirms the formation of a new phase i.e. Co-crystals. In the DSC thermogram, the distinct endothermic peak other than the melting peak of drug and CCF was

observed indicating the formation of co-crystal. Melting points of Co-crystals were much less than the Cilnidipine.

Table No. 3: CO-crystal formation determination by DSC

Sr. No.	Co-crystals	Method of co-crystal formation	Melting point of Drug(°C)	Melting point of Co-former(°C)	Melting point of Cocystal (°C)	Co-crystal formation by DSC
1	CLD- Benzoic acid (1:1)	Solvent evaporation	108.29	122.93	88.96	Yes

POWDER X-RAY DIFFRACTION (PXRD)

The PXRD pattern of a crystalline sample is considered as the fingerprint of its crystal structure. Every new crystalline material exhibits unique peaks indicative of reflections from specific atomic planes.

Characteristics reflections at 2θ values of 5.94, 10.92, 16.66, 20.06, 24.88 & 31.32 were observed for Cilnidipine. CLD- Benzoic acid prepared by solvent evaporation showed unique crystalline peaks at 2θ 6.02, 8.28.

Co-crystals of cilnidipine with citric acid also show some unique reflections but peak intensities were high. This might be the reason for the low aqueous solubility of this co-crystal.

Co-crystal of cilnidipine with sodium saccharine shows one unique reflection which is absent in both drug and coformer.

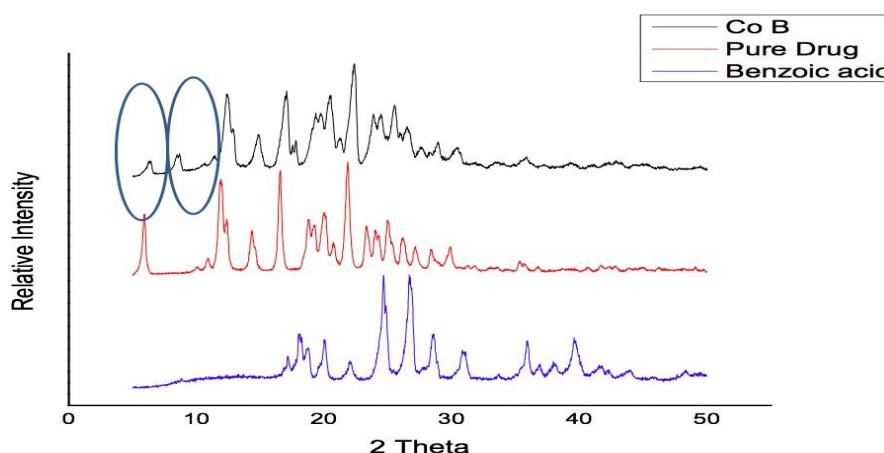


Figure No. 9: PXRD pattern of Co-crystals of CLD-Benzoic Acid

Citation: ASHISH V. THER et al. *Ijppr.Human*, 2020; Vol. 19 (1): 739-754.

PREFORMULATION CHARACTERIZATION OF CILNEDIPINE BENZOIC ACID COCRYSTALS

After evaluating all the parameters, CLD- benzoic acid co-crystals proved as a superior candidate than pure drug for further formulation development.

Table No. 4: Pre-formulation characterization of selected co-crystals

Sr. No.	Drug	Bulk Density gm./cm ³ (Mean± SD)	Tapped Density gm./cm ³ (Mean± SD)	Carr's Index % (Mean± SD)	Hausner Ratio (Mean± SD)	The angle of Repose (θ)(Mean± SD)
1	Cilnidipine	0.45±0.01	0.56±0.01	25.19±1.38	1.24±0.015	32°61'
2	CLD-Benzoic acid	0.48±0.02	0.55±0.038	13.92±2.378	1.16±0.034	28°76'

DRUG CONTENT

The drug content for Cilnidipine Benzoic acid co-crystal was found to be 95.02%.

DISSOLUTION STUDIES

Percent drug release from Cilnidipine Benzoic co-crystal was determined by the DBK dissolution apparatus.

Table No. 5: Percent drug release from pure drug and co-crystals

Time (min)	Pure drug Cilnidipine (%)	CLD- Benzoic acid (co-crystals (%))
5	28.4	43.92
10	32.08	57.18
15	37.86	60.73
20	41.67	68.06
25	42.93	82.12
30	45.94	91.84

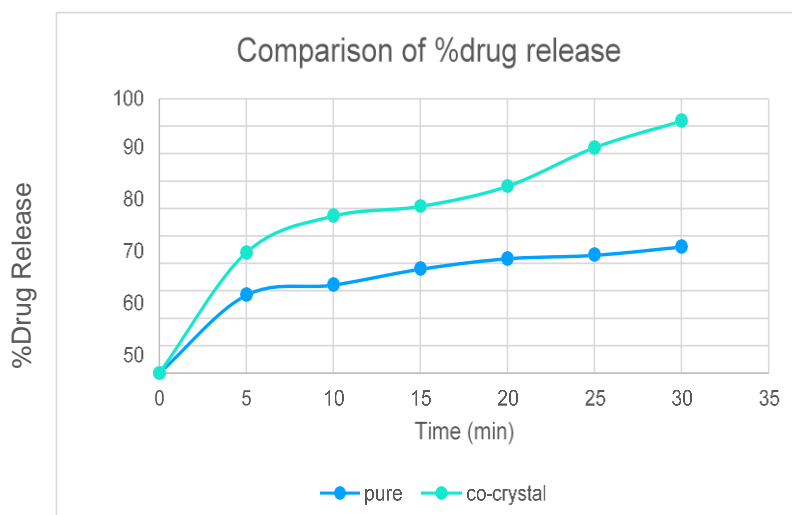


Figure No. 10: Comparative *in-vitro* dissolution test of CLD-Benzoic acid with pure drug Cilnidipine

CONCLUSION

Cilnidipine was crystallized, and the crystal structure was determined. Co-crystals with Benzoic acid, Citric acid, Sodium saccharine, Tartaric acid, and Boric acid were prepared using both solution and solid-state methods. It was found that Benzoic acid formed co-crystal in (1:1) stoichiometric ratio with Cilnidipine using both solution and solid-state methods, depending on their structures and the ability to form hydrogen bonds. The formation of a new phase was confirmed from DSC and PXRD analysis. The co-crystal showed enhanced solubility, flowability, and dissolution rate. CLD-Benzoic acid co-crystal (1:1) prepared by the solvent evaporation method passes all characterization tests of co-crystal determination thus it concludes the formation of co-crystal. By comparing pre-formulation characterization of pure Cilnidipine with prepared CLD-Benzoic acid co-crystals, co-crystals show improved pre-formulation properties. A comparison of the *in-vitro* dissolution of CLD-Benzoic acid co-crystal with pure Cilnidipine shows improvement in the dissolution rate of co-crystals. Thus based on formulation and development and its outcome co-crystal formation is another alternative to improve poor aqueous solubility.

REFERENCES

1. Scott L Childs, et al. 'Crystal Engineering Approach To Forming Cocrystals of Amine Hydrochlorides with Organic Acids. Molecular Complexes of Fluoxetine Hydrochloride with Benzoic, Succinic, and Fumaric Acids'. *Journal of the American Chemical Society* 2004; 126: 13335–13342.
2. Blagden N. et al 'Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates'. *Advanced Drug Delivery Reviews*. 2007; 59: 617–630.
3. Schultheiss N. Newman A. 'Pharmaceutical Cocrystals and Their Physicochemical Properties'. *Crystal Growth and Design*. 2009; 9(6): 2950–2967.
4. Blagden, N. et al. (2007) 'Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates', *Advanced Drug Delivery Reviews*. 2007; 59: 617–630.
5. Bavishi DD. Borkhataria CH. ('Spring and parachute: How cocrystals enhance solubility', *Progress in Crystal Growth and Characterization of Materials*. Elsevier Ltd. 2016; 62: 1–8.
6. Friščič T. Jones W. 'Recent advances in understanding the mechanism of cocrystal formation via grinding', *Crystal Growth and Design*. 2009; 9(3): 1621–1637.
7. Yadav A. et al. 'Co-crystals: A novel approach to modify physicochemical properties of active pharmaceutical ingredients'. *Indian Journal of Pharmaceutical Sciences*. 2009; 71(4): 359–370.
8. Qiao, N. et al. (2011) 'Pharmaceutical cocrystals: An overview', *International Journal of Pharmaceutics*. Elsevier B.V. 2011; 419: 1–11.
9. Desiraju GR. 'Supramolecular Synthons in Crystal Engineering—A New Organic Synthesis', *Angewandte Chemie International Edition in English*. 1995; 34: 2311–2327.
10. La'szlo Fa'bia'n. 'Cambridge structural database analysis of molecular complementarity in cocrystals', *Crystal Growth and Design*. 2009; 9(3): 1436–1443.
11. Issa N. Towards more Efficient Screening of Pharmaceutical Cocrystals. 2011: 1-232.
12. Gadade DD. et al. ('Cocrystallization of Etodolac: Prediction of Cocrystallization, Synthesis, Solid State Characterization and In Vitro Drug Release', *Marmara Pharmaceutical Journal*. 2017; 21: 78–88.
13. Basavoju S. Boström D. Velaga SP. 'Indomethacin-saccharin cocrystal: Design, synthesis and preliminary pharmaceutical characterization'. *Pharmaceutical Research*. 2008; 25(3): 530–541.
14. Blagden N. et al. 'Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates', *Advanced Drug Delivery Reviews*. 2007; 59: 617–630.
15. RP S., R.P J. 'Preparation and In-Vitro Characterization of CrystalloCo-Agglomerates of Cilnidipine'. *Journal of Medical and Pharmaceutical Innovation*, 2016; 3(13): 12–20.
16. Fukte SR, Wagh MP, Rawat S. 'Innovare Academic Sciences Review Article COFORMER SELECTION : AN IMPORTANT TOOL IN COCRYSTAL FORMATION', *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014; 6 (7): 9–14.
17. Cysewski, P. and Przybyłek, M. 'Selection of effective cocrystals former for dissolution rate improvement of active pharmaceutical ingredients based on lipoaffinity index', *European Journal of Pharmaceutical Sciences*. Elsevier B.V. 2017: 1–20.