# SOLID STATE MODIFICATION BY CO-CRYSTALLIZATION OF SELECTED DRUG

A Thesis submitted to Gujarat Technological University

for the Award of

**Doctor of Philosophy**

in

**Pharmacy**

**Ms. Shah Priyal Kalpeshkumar**

Enrollment No. 199999901528

Under supervision of

**Dr. Anuradha K. Gajjar**

Professor and HOD,
Department of Pharmaceutical Chemistry and Quality Assurance
L. M. College of Pharmacy, Ahmedabad – 380009
Gujarat, India


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**AHMEDABAD**

**[October 2024]**

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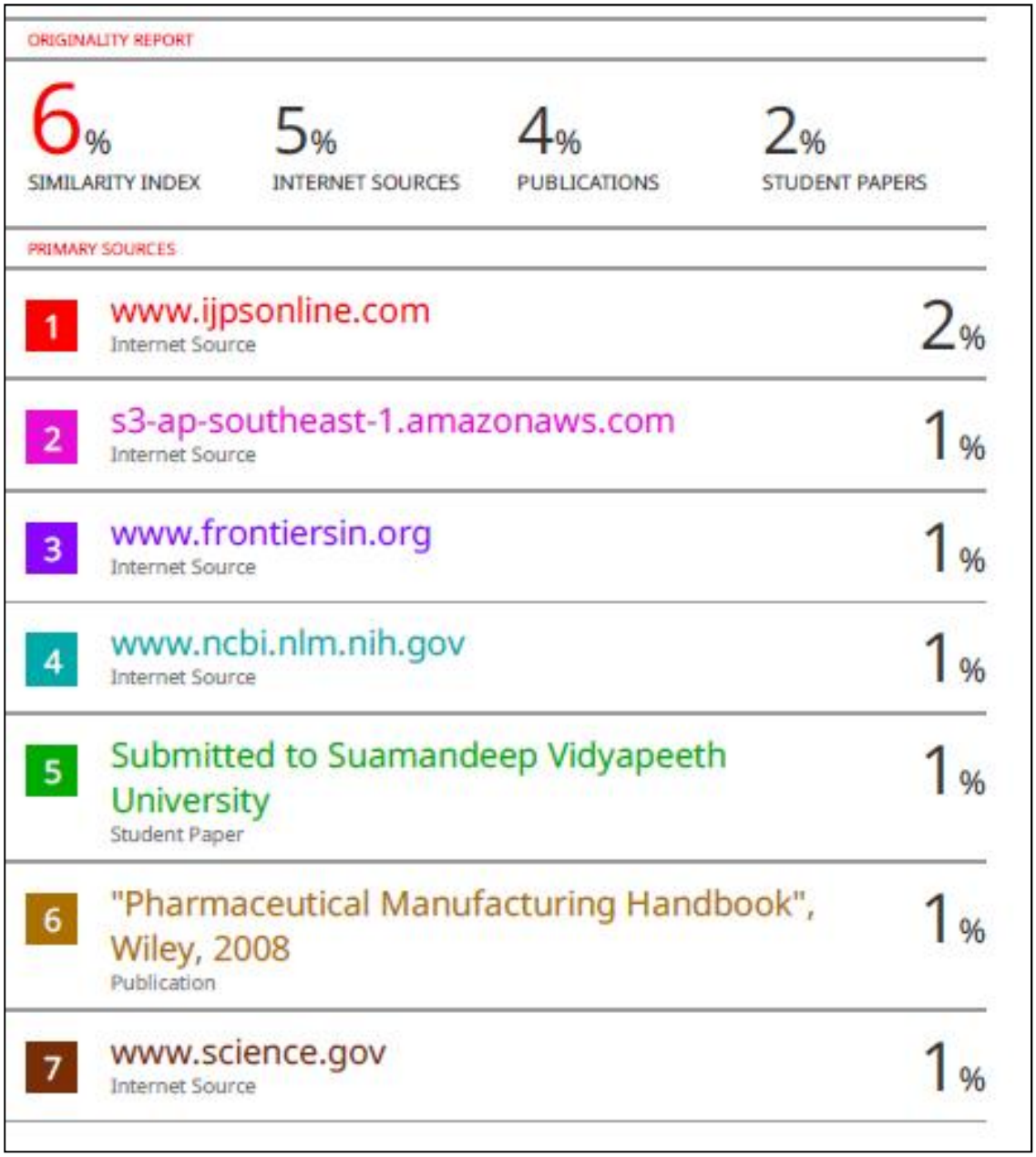

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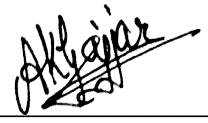
Signature of Supervisor:  Date: 08 Oct-24

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## ABSTRACT

A significant percentage of drugs, around 60-70 %, fall under BCS Class II, indicating their poor solubility. The cocrystal formation presents a promising solution to enhance drug solubility by modifying the crystal structure, thus potentially improving bioavailability. Fimasartan Potassium Trihydrate (FPT) is an antihypertensive molecule classified as BCS-II, presenting low solubility and high permeability. To address the solubility and bioavailability challenges associated with such compounds, cocrystal technology was employed in this study. A cocrystal of FPT with L-Proline (FPT-LP) was successfully obtained using the solvent evaporation method at a stoichiometric ratio of 1:2. The scalability of this technique was demonstrated through the Supercritical Fluid Extraction method for technology transfer and scale up. In silico studies employed encompassed in-silico cocrystal screening, the pKa rule, cocrystal structure analysis, Hirshfield Surface Analysis (HSA), and Surface Electrostatic Potential (SEP). This revealed the binding mechanism of FPT and the tendency of coformers to form cocrystals. This involves intermolecular hydrogen bonding (amino-nitro) and π-π stacking. The developed cocrystals were characterized comprehensively using various techniques like FTIR, SEM, TEM, DSC, PXRD, SCXRD, RP-HPLC, and U.V. visible spectrophotometer. The relative crystallinity study showed that the developed cocrystals are crystalline (66.98 %). The solubility studies showed eight fold increase in the solubility of FPT-LP compared to FPT. In-vivo studies were conducted on rats using the LC-MS/MS method that demonstrated a remarkable 88.88 % increase in bioavailability of FPT-LP compared to FPT. The developed cocrystals were successfully formulated into capsule dosage form, exhibiting drug release of 99.95 % within 60 minutes in phosphate buffer-6.8. Stability studies were conducted via Dynamic Vapor Sorption, Photostability and Accelerated stability studies for FPT-LP cocrystal and the developed capsule formulation which confirmed the stability of the cocrystal. Additionally, In-vitro In-vivo correlation (IVIVC) analysis showed a direct point-to-point relationship between % Drug Release and Absorption Rate (Level-A correlation). The development of this cocrystal offers substantial benefits to society by enhancing drug bioavailability, and decreasing the FPT dose. It also provides advantages to the industry through a facile and scalable

manufacturing method. Moreover, the work is protected under patent, ensuring regulatory advantages.
*Keywords:* Fimasartan; L-Proline; co-crystallization; bioavailability; supercritical fluid extraction; IVIVC; cocrystal; capsule

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*-Priyal Shah*

*'Individually, we are one drop. Together, we are an ocean' says Ryunosuke Satoro. This thesis embodies the collective dedication and expertise of "**women team**"; comprising the research scholar, supervisor, DPC members and the thesis reviewers, who have joined together to deliver a comprehensive and enriching research study that benefits the society.*

## Table of Contents

## List of Abbreviations

| Short-form | Long-form |
|---|---|
| µg/mL | Microgram per milliliter |
| µl | Microlitre |
| ACN | Acetonitrile |
| API | Active Pharmaceutical Ingredient |
| AUC | Area Under the Curve |
| BCS | Biopharmaceutical Classification System |
| CAS | Chemical Abstract Service number |
| CC | Cocrystal |
| CCS | Cross Carmallose Sodium |
| CDSCO | Central Drug Standard Control Organization |
| Cl | Clearance |
| Cm | Centimeter |
| $C_{max}$ | Concentration Maxima |
| $CO_2$ | Carbondioxide |
| CSD | Cambridge Structure Database |
| DPC | Doctoral Progress Review Committee |
| DSC | Differential Scanning Calorimetry |
| DVS | Dynamic Vapour Sorption |
| EDTA | Ethylenediamine Teraacetic acid |
| ESI | Electron Spray Ionization |
| F | Formula |
| FPT | Fimasartan Potassium Trihydrate |
| FPT-LP | Fimasartan Potassium Trihydrate – L-Proline Cocrystal |
| FTIR | Fourier Transform Infrared |
| gm | Gram |
| GRAS | Generally Regarded as Safe |
| h | Hour |
| HCTZ | Hydrochlorothiazide |
| HPLC | High Performance Liquid Chromatography |
| HSA | Hirshfield Surface Analysis |
| IAEC | Institutional Animal Ethics Committee |
| ICH | International Conference on Harmonization |
| IUPAC | International Union of Pure and Applied Chemistry |

| | |
|---|---|
| IVIVC | In Vitro In Vivo Correlation |
| Kcal/mol | Kilocalorie per mole |
| LC-MS/MS | Liquid Chromatography-Mass Spectrometry |
| LOD | Limit of Detection |
| Log P | Partition Coefficient |
| LOQ | Limit of Quantification |
| LP | L-Proline |
| M | Molar |
| MCC | Microcrystalline Cellulose |
| MEPS | Molecular Electrostatic Potential Surfaces |
| mg | Milligram |
| Mg. Stearate | Magnesium Stearate |
| mg/mL | Milligram per milliliter |
| Min | Minute |
| mL | Milliliter |
| mm | Millimeter |
| NLT | Not Less Than |
| Nm | Nanometer |
| NMT | Not More Than |
| º C | Degree Celsius |
| pH | Negative Logarithm of the Hydrogen Ion Concentration |
| PK | Pharmacokinetic |
| pKa | Ionization Constant |
| PM | Physical Mixture |
| psi | Pound force per Square Inch |
| PXRD | Powder X-ray Diffraction |
| RH | Relative Humidity |
| RPM | Revolution per Minute |
| RSD | Relative Standard Deviation |
| Rt | Retention Time |
| SCXRD | Single Crystal X-ray Diffraction |
| SD | Standard Deviation |
| SEM | Scanning Electron Microscopy |
| SEP | Surface Electrostatic Potential |
| SFE | Supercritical Fluid Extraction |

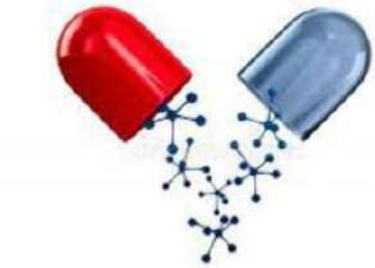
| | |
|---|---|
| SSG | Sodium Starch Glycolate |
| $T_{1/2}$ | Half life |
| TEM | Transmission Electron Microscopy |
| $T_{max}$ | Time Maxima |
| TOF | Time of Flight |
| USFDA | United States of Food and Drug Administration |
| UV | Ultra Violet |
| v/v | volume/volume |
| $V_{ss}$ | Steady State Volume |
| $V_z$ | Volume of Distribution |
| λmax | Lambda max |

# List of Figures

## List of Tables

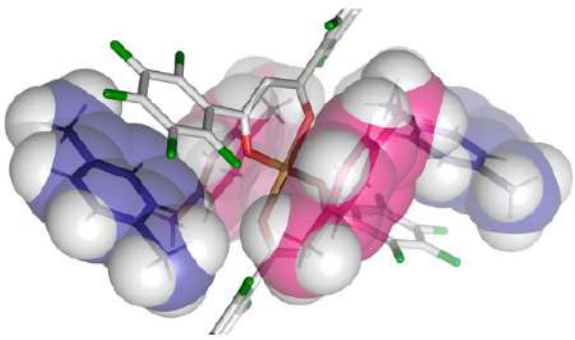
## Annexure

| | |
|---|---|
| Annexure I | IAEC Approval Letter |
| Annexure II | Patent Application |
| Annexure III | Fellowship Letter |
| Annexure IV | Summary of Compliance Report (DPC - I to DPC - VI) |
| Annexure V | List of Publications |
| Annexure VI | List of Conference Presentations |
| Annexure VII | List of Major Participations |



# Chapter-1

# Introduction



# CHAPTER-1
# Introduction

The recent trend states that approximately 40 % of currently marketed drugs and up to 70 % of compounds currently under development follows Biopharmaceutical Classification System (BCS) Class II – that is low solubility and high permeability. The amount of drug reaching the systemic circulation becomes insufficient with low solubility hence lesser amount of drug reaches the site of action, leading to poor bioavailability. Various solubility enhancement approaches that include physical and chemical modification of drug and other methods like particle size reduction, nano suspension technology, salt formation, cocrystal formation etc. have been tried to overcome these issues.[1]

## 1.1 Pharmaceutical Solids - States

The Active Pharmaceutical Ingredients (APIs) mostly are formulated as oral solid dosage forms, in the form of capsules and tablets as they offer a number of advantages like formulation strategy, scale up, low cost of production, stability and better patient compliance.[1] The solid state of an API is of critical importance during the development of an oral dosage form. This has a larger impact on the physical stability, hygroscopicity, dissolution rate, solubility which ultimately leads to increased bioavailability. Solid states of API can exist as crystalline forms (polymorphs, hydrates, solvates, cocrystals, and salts) and amorphous forms(Fig-1.1).[2] Crystalline forms are the solids that exist as a three-dimensional long-range order in which the structural units or unit cells are regularly repeated indefinitely in three dimensional space. Each crystal can be further classified on the basis of dimensions and the individual angles of the unit cell.[3] Crystal Polymorphs is the ability of a compound to exist as more than one distinct crystal form that has same chemical composition but different internal packing structures.[4] Polymorphism is further categorised as: packing, conformational, tautomeric and synthon polymorphism.[5] Solvates, are crystalline solids containing any solvent molecule incorporated into the crystal structure, either in stoichiometric or non stoichiometric amounts. These are also termed as pseudo polymorphs. If water is incorporated as the solvent molecule, it is termed as hydrate[6]. The presence of solvates exhibits differences in the dissolution rate and solubility in comparison to

the non-solvated form of a drug. The API itself often has an alkaline/acidic group, and the addition of a suitable acid/alkali material may result in the salt formation.[7]

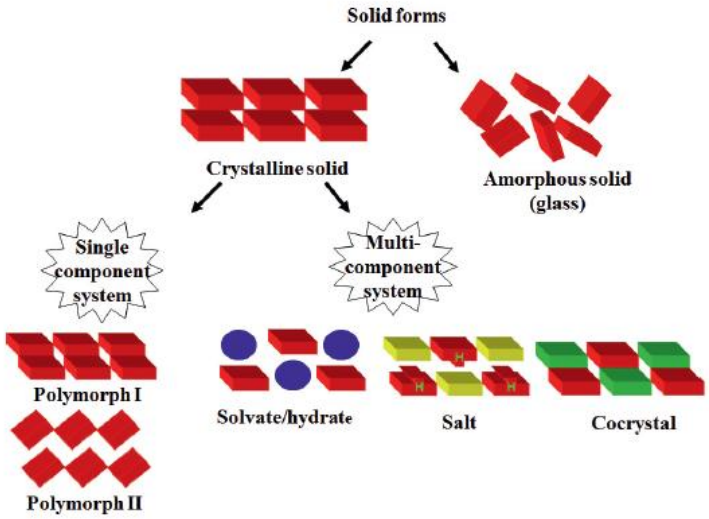



Figure-1.1: Solid State forms of a drug substance[1]

## 1.2 Cocrystals as a Solid Form

### *1.2.1 Cocrystal definition*

The term cocrystal was first coined by Etter. He also stated the hydrogen bonding rules for the formation of organic crystal, [8,9] while, Desiraju was the first to give the supramolecular synthon concept of hydrogen bond formation in crystal structures.[10] In 2004, the new era of crystal engineering and cocrystal formation began which showed that the crystalline materials are capable of altering the physicochemical properties of APIs without altering the physiological properties.[11] Duggirala and coworkers differentiated the cocrystals obtained from the physical mixture and different types of coformers on the basis of the molecular and ionic nature of the coformer.[12]The term cocrystal (or cocrystal) is defined as crystalline materials composed of two or more different molecules, typically active pharmaceutical ingredient (API) and cocrystal formers (coformers), in the same crystal lattice in a specific stoichiometric ratio (Fig-1.2)[13]

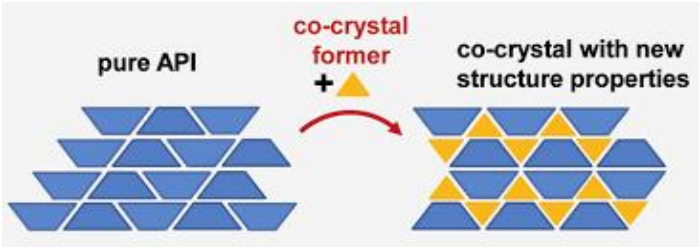



Figure-1.2 Cocrystal Formation[2]

### *1.2.2 Structural diversity of cocrystals*

The Cocrystals can either be in the form of weak acid, base and /or even neutral theoretically be formed from weakly acidic, basic or even neutral molecules.[14] The coformers for the formation of cocrystal can either be an API or excipient. Hence, a single API can form variety of cocrystals with unique structural and physical properties. Cocrystals also exhibit diversity in stoichiometric ratios i.e. the drug and the coformer can be present in different ratios like 1:1, 1:2, 2:1 and so on.[15] For example U-maleic acid cocrystals were prepared with the stoichiometric ratios like 2:1, 1:1, and 1:2.[16] Studies show that during the formation of cocrystals the API tend to crystallize as hydrates and as solvates[17] The crystal engineering strategy shows structural diversity of cocrystals that helps in improving the variety of drug solid forms. This feature offers opportunities for tuning various properties of the drug. The structural diversity of multicomponent crystals can be predicted by computational methods.[18]

## 1.3 Applications of Cocrystals

The physicochemical properties altered by the formation of cocrystal stands out from all the advantages that exist in the stable crystalline form.[19] The vital factor that plays an important role in altering the physicochemical properties is the coformer.[20] The cocrystals can also be for non-ionizable APIs and for complex drugs with varied functional groups.[21] The most important advantage for the formulation of cocrystals is the shortening of the drug development timeline of APIs with higher yield and lower costs. The developed pharmaceutical cocrystals are structurally different from their API and hence have the option of patenting existing API cocrystals as a new crystal form. There are various examples of pharmaceutical cocrystals available as marketed formulations. **Suglat®** a diabetes mellitus-2 formulation (Ipragliflozin – L-Proline cocrystal) increases the stability against hydrate formation, manufactured by Astellas Pharma and Kotobuki Pharmaceutical. **Entresto®** by Novartis, is used for reducing the risk of heart failure is a Valsartan-Sacubitril cocrystal that leads to improved pharmacokinetics and bioavailability of Valsartan. **Steglatro®** is a medication for type-2 diabetes mellitus (Ertugliflozin - Z-Pyroglutamic acid cocrystal) which gives improved stability. **Depakote®** is a cocrystal of Valproic acid – Valproate sodium, used to treat epilepsy that has more solid phase stability and less hygroscopicity.

**Lexapro®** is a medicine for depression (Escitalopram-Oxalate cocrystal) that improved stability. **Viagra®** by Pfizer is used for the treatment of erectile dysfunction, that comprised of sildenafil citrate – acetyl salicylic acid.[21] Various physicochemical properties of drugs like melting point, tabletability, solubility, stability, bioavailability, permeability may be altered by the formation of a pharmaceutical cocrystal.

Melting Point: The findings states that the melting point of cocrystals and coformers are interrelated, but the melting point and solubility are not linearly correlated.[22] So, to stabilize the thermolabile API, coformer with high melting point should be selected. This results in higher melting point of the cocrystal without altering the solubility.[23]

Tabletability: The cocrystal formation may directly affect the crystal packing, tabletability and compaction, which are important parameters in the preformulation study. In some cases, cocrystals have higher tabletability than either pure drug or coformers.[24]

Example: The paracetamol cocrystals (with oxalic acid) showed better compaction behavior than that of a pure drug. Resveratrol cocrystal with 4-aminobenzamide and isoniazide as coformers showed enhanced tabletability properties.[25]

Solubility: Solubility is a major challenge for formulation scientist as 40% of the new drugs developed are practically insoluble in water. Various techniques are used to increase the solubility such as salt formation, solid dispersion, particle size reduction, cocrystallization and so on.[26]

Example: The ketoconazole drug showed less solubility in comparison to ketoconazole cocrystals (increased by 100 folds) while its salt formation lead to increase in solubility by 53 folds.[26] The cocrystal of the antitumor drug 6-mercaptopurine showed twice the solubility of the drug alone. [27]

Stability: The stability studies play a vital role in the development of new dosage formulations. Various stability issues like chemical stability, solution stability, relative humidity, thermal stability and photostability are studied in preformulation studies.[28,29]

Example: Indomethacin-saccharin cocrystals showed good stability to relative

humidity study by showing lower water sorption.[29] Theophylline cocrystals with oxalic acid was studied at varied % RH like 40, 73 and 98 % for 1, 3 and 7 weeks. The results showed improved physical and stability properties as it avoided the hydrate formation,[30] L-883555, an inhibitor of phosphodiesterase IV cocrystal with tartaric acid was studied for different stoichiometric ratios ranging from 0.3 : 1.0 to 0.9 : 1.0. Cocrystals with stoichiometric ratio with 0.5:1.0 was found to be most stable, as the acid content occupied the channels in the crystals and establish multiple binding modes that showed better thermal stability.[31]

Bioavailability: Crystal engineering is primarily used to design and synthesize pharmaceutical cocrystals with enhanced aqueous solubility and oral bioavailability as low bioavailability is the major issue with BCS Class II and IV molecules. The oral bioavailability of baicalein-nicotinamide cocrystals showed peak plasma concentration ($C_{max}$) as 2.49-fold higher than the pure drug in rats.[32]

Permeability: The permeability of the API is a major challenge with BCS class-III drugs. The cocrystal technique helps in easing the permeation by the selection of appropriate coformer.[33]

Example: 5-fluorouracil cocrystal showed increase in the permeability with different coformers like 3-hyroxybenzoic acid, 4-aminobenzoic acid and cinnamic acid. Hydrochlorothiazide - succinamide cocrystals showed increase in the permeability when studied by using Franz diffusion cells. The amount of drug flux in cocrystal was higher as compared to pure drug.[33]

**Advantages:** In pharmaceuticals, one of their primary benefits lies in enhancing the solubility of poorly water-soluble drug compounds. This solubility improvement can lead to increased drug bioavailability, allowing for lower dosages and reduced side effects, while maintaining therapeutic efficacy. Furthermore, cocrystals enable the tailoring of a compound's physical and chemical properties, including stability and dissolution rates, facilitating optimized drug formulations and extended patent life, which guards against generic competition.[4]

*Enhanced Drug Solubility:* Cocrystals play a vital role in enhancing the solubility of poorly water-soluble drug compounds, thereby increasing drug bioavailability. This enhancement enables the administration of lower dosages, resulting in

reduced side effects while maintaining therapeutic efficacy.[5]

*Tailored Physical and Chemical Properties:* Cocrystals allow for the customization of a compound's physical and chemical properties, including stability and dissolution rates. This capability facilitates the development of optimized drug formulations and extends patent life.[4]

*Improved Stability:* Cocrystals contribute to enhanced stability, both chemical and physical, leading to an extension of the product shelf life. This improved stability ensures the maintenance of drug efficacy over an extended period, benefiting both patients and pharmaceutical manufacturers.[4]

*Economic Advantages:* Utilizing cocrystals in drug development and manufacturing processes offers economic benefits, including cost savings. The streamlined development process and improved manufacturing efficiency contribute to reduced expenses, making drugs more accessible and affordable.[6]

*Safety and Versatility:* Cocrystals offer the potential to create safer pharmaceutical products with lower toxicity profiles. This versatility provides innovative solutions to formulation challenges and enhances the optimization of existing compounds across different industries.[5]

**1.4 Strategy for Cocrystal Formation**

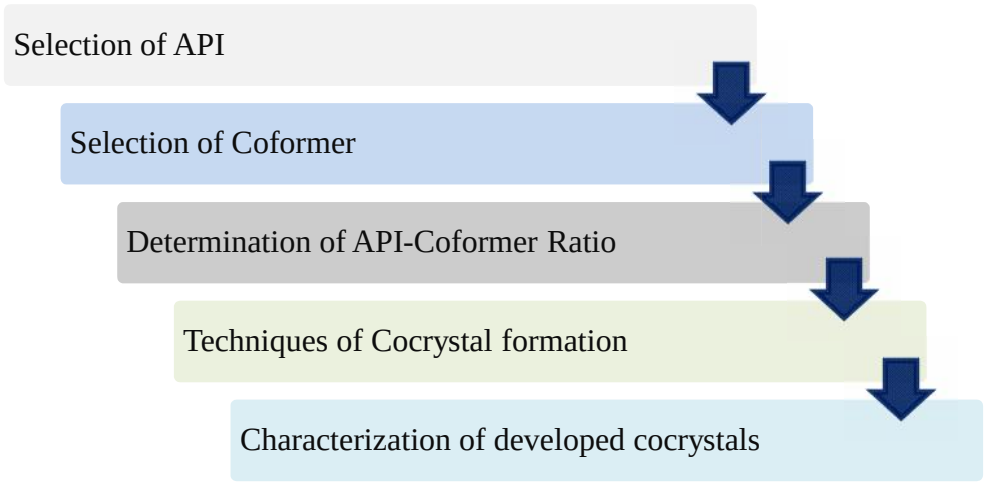



Figure-1.3: Strategy for cocrystal formation[5]

The strategy for cocrystal formation involves several sequential steps aimed at optimizing the combination of the active pharmaceutical ingredient (API) and the coformer (Fig-1.3). The initial and crucial step is selecting the appropriate API,

followed by determining the most suitable coformer, which is essential for successful cocrystal formation with its desired activity. To study the hydrogen bond formation between the API and coformer, various computational in-silico techniques are employed. These methods provide valuable insights into the selection of potential coformers that leads to cocrystal formation.[4] Once the coformer is identified, the API and coformer stoichiometric ratio are determined through a series of trials and errors using different techniques to achieve optimal cocrystal formation. The developed cocrystal is subjected to comprehensive characterization using sophisticated techniques such as Powder X-ray Diffraction (PXRD), Single-Crystal X-ray Diffraction (SCXRD), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).[5] These analyses confirm the formation of the cocrystal and provide information about its crystallinity (Fig-1.3) Moving forward, pharmacokinetic studies are conducted to assess the cocrystals effect in comparison to the API. These studies help in understanding the change in pharmacokinetic properties and calculating bioavailability. Later, the cocrystals are tailored for dosage form preparation, stability and shelf life studies.[3]

### 1.4.1 *Selection of Coformers*

The coformer can either be another drug or excipient or aminoacid.[34] There are thousands of substances that could be used as potential coformers that is stated by USFDA in GRAS.[35] The only important criteria is it should be non-toxic with no adverse effects with the presence of at least one functional group. Coformer selection plays the vital role in the designing and screening of cocrystals. The selection of coformers majorly depends on knowledge-based methods and experimental or trial and error methods. The knowledge based approach includes various computational techniques like hydrogen bonding propensity, supramolecular compatibility by Cambridge Structure Database (CSD), pKa based models and the conductor-like screening model for real solvents (COSMO-RS), Hansen solubility parameter, virtual cocrystal screening (based upon molecular electrostatic potential surfaces-MEPS), thermal analysis, measuring saturation temperature, Kofler contact method and matching.[36]

**Hydrogen bonding propensity:** In the cocrystal formation API and coformers interact with each other with a non-covalent bond, like hydrogen bonds and van der Waal forces.[37] Amongst these, the hydrogen bonding between the API and the coformer plays an important role in the formation of cocrystals.[38] Quantitative measurements of hydrogen bond formation between the donor and acceptor functional groups present is studied through CSD.[39]CSD is a tool that to facilitate statistical analysis of packing patterns and provide information on common functional groups. This is used to identify appropriate cocrystal-forming pairs through computer-based approach to reduce research time and experimental costs.[37]

**pKa rule:** ΔpKa helps in predicting as well as identifying the formation of a cocrystal and/or salt. This is simple yet time consuming method for cocrystal determination.[40] The solvents with the highest proton transfer values were identified between the acid and the base and it is calculated by ΔpKa = [ΔpKa(base) – ΔpKa(acid)]. ΔpKa less than 0 indicates cocrystal formation, where as values more than 2 or 3 indicates the formation of salts.[41]

**Hirshfield Surface Analysis (HSA):** It is a tool for gaining insight into the intermolecular interaction of the API and the coformer. It majorly helps in identifying the crystal packaging behaviour. The size and shape of Hirshfeld surface allows the qualitative and quantitative investigation. CrystalExplorer software is used to determine HSA.[42]

**Electrostatic Surface Potential (ESP):** Electro Static Potential is an important physicochemical property of a compound as it provides information about the surface charge density distribution and molecular reactivity. ESP illustrates charge distribution three dimensionally. The software has a database classified into functional groups having the site of interaction in the cocrystal as primary minima and maxima based on surface charge density. Similarly, the various functional groups in the coformers are classified as secondary maxima and minima. The plane of the API rotates because of repulsion between the primary minima site and coformer secondary minima site. Hence, the maxima sites of both API and coformer come in contact with each other. The major application of ESP is providing insights into the intermolecular binding of functional groups on the basis of surface charge.[43]

### 1.4.2 *Techniques for Cocrystal Formation*

There are various techniques available for the preparation of cocrystals. They are mainly divided into, traditional methods and sophisticated methods which are further classified into various techniques (Fig-1.4).[44]

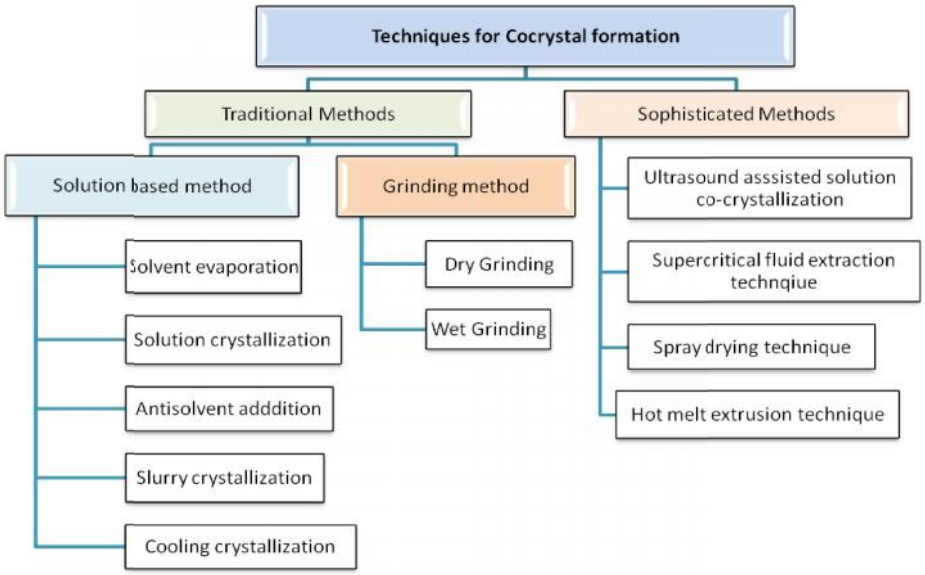



Figure-1.4: Techniques for Cocrystal Formation

**Solvent Evaporation Technique**: The API and coformer are dissolved in appropriate solvent and the solution is allowed to evaporate slowly. This method is used when API and coformer are thermodynamically stable.[44]

**Solution Crystallization Technique**: The API and coformer are dissolved in boiling solvent with stirring with reduction in solvent volume. Solvent is allowed to cool rapidly. Cocrystals are separated by filtration or drying in oven. This method is used when API and coformer are stable under both hot and cold conditions.[45]

**Antisolvent Crystallization Technique:** The API and coformer are dissolved in different suitable solvents and are homogenized. These solutions are added to the solvent which precipitates the API and coformer. The rate of addition of the solvent plays a vital role. Also, combining anti solvent cocrystallization and cooling method gives better results for cocrystal formation.[46]

**Slurry crystallization Technique**: Slurry is formed when API and coformer are mixed with a suitable solvent. The excess solvent is decanted and the cocrystals are dried. This method is used when the API and coformer, both show good solution stability.[47]

**Cooling Crystallization Technique**: Super saturated solutions of the API and coformer in suitable solvents are mixed and then cooled. This method relies on the temperature-dependent solubility change to achieve cocrystal formation.[47]

**Dry Grinding and Wet Grinding Technique:** The API and conformer are mixed together and ground in a mortar without any solvent (dry grinding) and with a suitable solvent (wet grinding). Real time monitoring of the cocrystal formation is the greatest challenge in this method**.**[48]

**Ultrasound assisted solution Cocrystallization Technique:** The API and coformer are dissolved together in a solvent in a sonoreactor to form a turbid solution. Cold water is supplied continuously to avoid fragments in nucleation and the solution is kept for drying overnight. Sonication can lead to increase in the nucleation rate and alter the phase diagram as well.[49]

**Supercritical Fluid Extraction (SFE) Technique:** SFE is the most advantageous technique for cocrystallization as it gives the highest quality of cocrystals. The most commonly used supercritical fluid is $CO_2$; the advantages of using this fluid is to reduce the processing steps, being eco-friendly solvent, well-finished products, a greater tendency for solubility, and less product degradation. The supercritical $CO_2$ acts as a solvent that leads to the nucleation growth and formation of co crystals by intermolecular interactions between the API and coformer. Solubility can be altered by adjusting the temperature and pressure conditions (of $CO_2$). The advantage of this technique is that it prevents the formation of solvates and hydrates in the cocrystals because of the absence of water in its preparation. It is limited to single-component cocrystal formation.[50]

Spray drying technique: The API and coformer are dissolved in a solvent and solution/suspension is formed, which is subjected to spraying in the hot air stream to evaporate the solution. This technique is not useful for thermodynamically unstable API and/or coformer. [45]

**Hot melt extrusion Technique:** The API and coformer are heated with intense mixing without the use of any solvent. The formation of the cocrystals occurs once the API and the coformer starts melting and this is the initiation phase of nucleation. The major drawback of this technique is both the API and coformer should be miscible in molten forms hence it cannot be used for thermolabile drugs.[51]

### *1.4.3 Characterization of cocrystals*

There are various techniques to characterize cocrystals like PXRD, FTIR, DSC, SCXRD, SEM and TEM. Each technique with its importance in cocrystal characterization is stated below.

**FTIR spectroscopy:** FTIR spectroscopy is employed to identify cocrystal formation and confirm the presence of specific interacting functional groups. It confirms the formation of a cocrystal by detecting shifts in absorption peaks or the emergence of new peaks in the spectrum. These changes indicate that the interacting molecules are in a cocrystalline arrangement. Quantification of cocrystal composition can be calculated by comparing the intensities of specific bands corresponding to the cocrystal components, that determines the relative quantities of each component in the cocrystal with API and physical mixture. FTIR characterizes intermolecular interactions, making it a crucial tool in determining the cocrystals in the initial phase of study.[52]

**Differential Scanning Calorimetry (DSC):** DSC is a critical tool for determining the cocrystal formation. It provides information on the thermal properties, stability, and behaviour under various conditions of cocrystals. DSC can accurately determine the melting point of cocrystals, aiding in their identification and characterization. It also reveals the heat flow associated with phase transitions, such as melting or recrystallization, providing insight into cocrystal stability and behaviour over a range of temperatures. Presence of impurities in cocrystals can also be detected by polymorphic transitions that may affect their properties through heat. DSC evaluates the compatibility of cocrystal components and assess potential interactions, ensuring product stability. Generally, when cocrystals are heated, the DSC scan shows an exothermic peak associated with cocrystal formation directly after an endothermic peak at a significantly higher temperature. In contrast, when a physical mixture incapable of cocrystal formation is heated, only a single endothermic peak associated with API melting appears on the DSC scan.[53]

**Thermogravimetric Analysis (TGA):** TGA is a valuable tool in cocrystal identification and characterization, particularly for assessing thermal stability, detecting impurities, and quantifying cocrystal purity. When used in conjunction

with other analytical techniques likes DSC and XRD it enhances the overall understanding of cocrystal properties and behaviour.[54]

X-ray Diffraction Study: XRD is primarily employed to determine the crystal structure of cocrystals. The XRD studies can be conducted by two ways: Powder XRD (PXRD) and Single Crystal XRD (SCXRD). By analyzing the diffraction pattern generated when X-rays interact with the cocrystal, the spatial arrangement of atoms or molecules in the crystal lattice can be determined. This information is fundamental in confirming the presence of a cocrystal. XRD can distinguish cocrystals from their individual components (that is API and coformer) and other solid forms. The unique diffraction pattern of a cocrystal serves as a fingerprint for its identification. This technique is essential for confirming the presence of cocrystals and knowing their structural properties.[55]

**Dissolution Studies:** Cocrystals are often designed to improve the solubility of poorly soluble drug compounds. Dissolution studies can demonstrate whether a cocrystal has the desired effect by comparing its dissolution rate to that of the API. A faster dissolution rate suggests improved solubility. Studying the dissolution kinetics of cocrystals provides information about the mechanisms governing the release of the cocrystal components. This can be important for understanding the dissociation and release of API from the cocrystal.[6]

**Solubility Studies:** Solubility studies are used in the initial stages of cocrystal formation, to identify potential coformers. The testing of different combinations of coformers and the compound of interest (API) at various concentrations and conditions helps in identifying systems in which cocrystals are likely to form based on changes in solubility behaviour. Solubility studies are crucial for optimizing cocrystal synthesis processes. It can determine the solvent, temperature, and concentration conditions that lead to the highest cocrystal yields. Solubility data aids in selecting the most suitable coformers for cocrystal formation. Coformers with complementary solubility profiles, maximize the chances of cocrystal formation with higher solubility. It also helps in establishing the optimum stoichiometric ratio of API and coformer with maximum yield.[7]

**Stability Studies:** Stability studies involve subjecting the cocrystal to various stress conditions such as temperature, humidity, and light. Changes in the

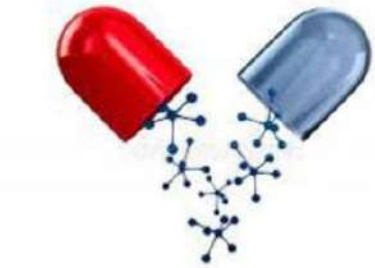
cocrystal's physical and chemical properties, including its crystallinity and spectral features, can be monitored to detect degradation or phase transitions. The absence of degradation or transformation indicates the stability of the cocrystal.[16]

### 1.5 Amino acids as Coformer

Amino acids are the organic components that combine to form proteins. These biomolecules are involved in several biological and chemical functions in the human body and are necessary for the growth and development of human beings. Amino acids are also generally recognized as safe (GRAS), which means that they have low toxicity. Structurally, amino acids are promising coformer candidates because of the presence of functional groups that have the tendency to form hydrogen bonds and increase stability through zwitterionic moieties that support strong interactions. Aminoacids can be used as coformers because they have amino and carboxylic acid groups that can act as donors and acceptors of hydrogen bonds. The studies prove that the range of amino acids were studied for the formation of cocrystals with varied API to solve or overcome limitations of varied physicochemical properties like improved stability and solubility problems.[56]

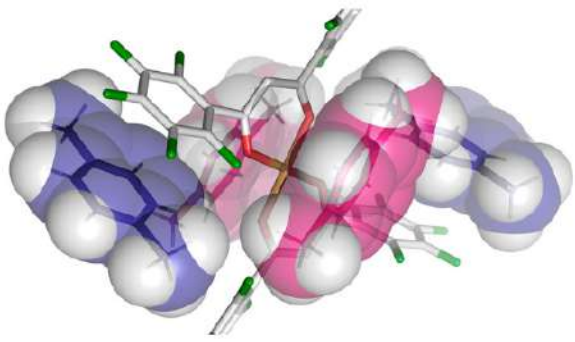
Amino acids can be broadly categorized into three groups based on the polarity of their side chains: non polar, polar, and charged (acidic or basic). Non polar amino acids have side chains that are hydrophobic and do not readily interact with water. The non polar amino acids with non polar (hydrophobic) side chains include Glycine, Alanine, Valine, Leucine, Isoleucine, Methionine and Proline.

Proline has a unique cyclic side chain that forms a ring structure, making it distinct from all other non polar amino acids.[56]



# Chapter-2

# Review of Literature



# CHAPTER - 2

# Review of Literature

The literature review was conducted with a purpose to provide a comprehensive understanding of the existing research and identify research gaps for further investigation. This helped in building a theoretical framework, validate research hypotheses and avoid duplication with enhanced credibility. The literature review is conducted in four major parts:

*Drug Profile of FPT:* This section provides a comprehensive overview of Fimasartan Potassium Trihydrate (FPT).

*Literature Review of cocrystals:* This part covers the techniques of preparation, identification, analytical methods and in-silico tools, involved in cocrystal formation and analysis.

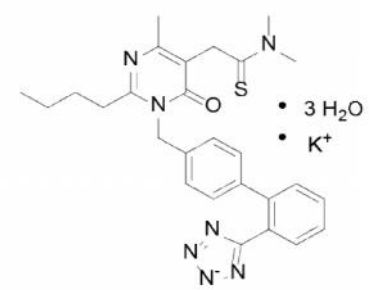
*Literature Review for class of drug:* This section focuses on the cocrystal formation of the sartan class of drugs, which served as a foundation for initiating the development of new cocrystals.

*Literature Review for analysis of FPT:* This part included various analytical methods and pharmacokinetic studies available related to FPT.

## 2.1 Drug Profile of Fimasartan Potassium Trihydrate

Fimasartan Potassium Trihydrate (FPT) is a novel angiotensin II receptor antagonist (ARB) that exerts its pharmacological effects by selectively blocking the angiotensin II receptor subtype 1 (AT1). This action results in vasodilation and inhibition of aldosterone secretion, leading to decreased blood pressure. FPT is indicated for the treatment of hypertension, offering an effective option for managing elevated blood pressure levels. With its favorable pharmacokinetic profile, including rapid absorption and elimination half-life suitable for once-daily dosing, Fimasartan provides convenient and reliable blood pressure control. Clinical studies have demonstrated its efficacy in reducing both systolic and diastolic blood pressure, with a well-tolerated safety profile. The detailed drug profile of FPT is stated in Table - 2.1.

Table-2.1: Drug profile for Fimasartan Potassium Trihydrate[57,58]

| | |
|---|---|
| Name | Fimasartan Potassium Trihydrate (FPT) |
| Structure |  |
| IUPAC Name | Potassium 5-(4'-((2-butyl-5-(2-(dimethylamino)-2-thioxoethyl)-4-methyl-6-oxopyrimidin-1(6H)-yl)methyl)-[1,1'-biphenyl]-2-yl)tetrazol-1-ide Trihydrate |
| Molecular Formula | $C_{27}H_{36}KN_7O_4S$ |
| Molecular Weight | 593.8 g/mol |
| CAS Number | 1020110-23-9 |
| Wavelength[52] | 260 nm |
| ***2.1.1 Physicochemical Properties*** | |
| Appearance | White powder |
| Solubility | 0.00542 mg/mL |
| BCS | Class II |
| Melting point | 155˚C |
| pKa value | 4.23(strongest acidic) 1.34 (strongest basic) |
| Log P | 4.03 |
| ***2.1.2 Pharmacokinetics of FPT*** | |
| Absorption | Absorbed rapidly with the $T_{max}$ 0.5 to 1.3 h and has minimal accumulation in the body |
| Metabolism | It is metabolized to FPT S-oxide, FPT N-glucuronide, oxidative desulfurized FPT (BR-A-557), and hydroxy-n-butyl FPT. |
| Half-life | 4 hours |
| Bioavailability | 18.6 % |
| Elimination | Bile excretion with half life of 7-10 h |
| ***2.1.3 Pharmacological & Therapeutic Properties*** | |
| Therapeutic category | Antihypertensive |
| Mechanism of action | FPT binds to Angiotensin II Type - I receptor to prevent vasoconstriction and reduces aldosterone secretion leading to a reduction in the blood volume. Together these effects produce an anti-hypertensive |

| | |
|---|---|
| | effect. |
| Dosage | 60-120 mg once daily |
| Therapeutic Use | For treatment of Hypertension |
| Adverse effect | Dizziness, Fainting and headache |
| Contraindications | Pregnancy and Lactation |
| Storage | Store below 30˚ C |
| CDSCO approval | September 2019 |
| USFDA approval | September 2010 |

### *2.1.4 Marketed Formulation*


Figure - 2.1 Marketed formulation of FPT[59]

FPT is available as a tablet with dosage of 60 mg and 120 mg by the name of Fimanta by Ajanta Pharma Ltd, India. Fig-2.1 shows the marketed formulation of FPT.[59]

## 2.2. Review of Literature for cocrystals

The literature review for cocrystals was conducted to get in depth knowledge about the cocrystals, its bonding technology, techniques, characterization and its regulatory importance (Table-2.2).

Table-2.2 Review of Literature for cocrystals

| Sr. No. | Title(Reference No) | Knowledge gained |
|---|---|---|
| 1 | Pharmaceutical Cocrystal: An overview[60] | Differences between cocrystals with salts, solvates and hydrates with the advantages of cocrystals. |
| 2 | Pharmaceutical cocrystals: walking the talk[61] | Some prominent examples of advantages of drug cocrystal depending on coformer selection. |
| 3 | Coformer Selection: An important tool in cocrystal formation[62] | Approaches like supramolecular synthon, Cambridge Structural Database (CSD), Hansen solubility parameter and knowledge of hydrogen bonding between coformer and API. |
| 4 | Creating Cocrystals: A Review of Pharmaceutical Cocrystal Preparation Routes and Applications[63] | Techniques for preparation of cocrystal with their advantages and disadvantage. |
| 5 | Cocrystal Formulation, | Various techniques and interpretation for the |

| | | |
|---|---|---|
| | Characterization, and Evaluation Study[64] | characterization of cocrystal |
| 6 | Detection of Cocrystal Formation Based on Binary Phase Diagrams[65] | Clarification on the relationship between exothermic peaks and cocrystal formation. |
| 7 | Advance methodologies for cocrystal synthesis[66] | Advancements include manufacturing of scalable, high quality cocrystals with enhanced physiochemical properties and appropriate drug-coformer selection. |
| 8 | Pharmaceutical Cocrystals: Regulatory, Strategic Aspects, Design and Development[67] | Significance of pharmaceutical cocrystals through regulatory perspectives focusing on United States and Europe. |

The review of literature for cocrystals gave a comprehensive overview on the formation of cocrystals, bonding chemistry, advanced techniques of preparation and characterization. This nurtured and molded the research work from identifying some basic techniques to scalable techniques for the cocrystal formation with a focus on its regulatory perspective.

**2.3 Review of Literature for class of drug**

The literature review suggested that there is no technique available for the formation of cocrystal of FPT. However, literature was available for other drugs of the same class. Hence the literature review was conducted on the sartan class of drugs (Table-2.3).

Table-2.3 Review of Literature for Cocrystals from the same class of drug

| Sr. No. | Name of the drug | Specifications |
|---|---|---|
| 1. | Telmisartan[68] | Coformer: Saccharin<br>Stoichiometric ratio: 1:1<br>Method of Preparation: Solvent Evaporation<br>Outcome: Improved solubility and increase in bioavailability (2 fold) |
| 2. | Olmesartan Medoximil[69] | Coformer: Hydrochlorothiazide<br>Stoichiometric ratio: 1:1<br>Method of Preparation: Solvent evaporation technique<br>Outcome: Improved solubility and minimized intermolecular drug-drug interaction |
| 3. | Valsartan[70] | Coformer: Succinic acid<br>Stoichiometric ratio: 1:1<br>Method of Preparation: Solvent evaporation method<br>Outcome: Improved solubility (10 fold) and improved micromeritic properties |

## 2.4 Review of Literature for FPT

The available analytical and bio analytical methods of FPT were studied for identification and characterization of FPT.

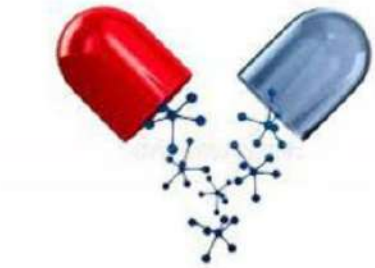
Table-2.4 Review of Literature for FPT

| Sr. No. | Title | Parameters |
|---|---|---|
| 1. | UV Spectrophotometric Method Development and Validation of Fimasartan Drug and its Tablet Formulation[71] | $\lambda_{max}$: 240 nm<br>Solvent: Water<br>Linearity Range: 0-20 ppm |
| 2. | Evaluation of stability and simultaneous determination of fimasartan and amlodipine by a HPLC method in combination tablets[72] | Column: C18 column (250 mm × 4.6 mm, 5 µm)<br>Mobile phase: Acetonitrile: 0.02 M mono potassium phosphate buffer (pH 2.2) [50:50 v/v]<br>Flow rate: 1.0 mL/min<br>Run time: 8 min<br>$\lambda_{max}$: 237 nm |
| 3. | Validated stability indicating RP-HPLC method for the determination of fimasartan in presence of degradation products[73] | Column: C18 column (250 mm × 4.6 mm, 5 µm)<br>Mobile phase: Acetonitrile: Phosphate buffer (pH -3) [50:50 v/v]<br>Flow rate: 1.0 mL/min<br>Run time: 15 min<br>$\lambda_{max}$: 262 nm |
| 4. | Simultaneous determination of fimasartan, a novel antihypertensive agent, and its active metabolite in rat plasma by liquid chromatography tandem mass spectrometry[74] | Instrument: API 4000 LC-MS/MS<br>Column: C18 column (50 mm × 2.6 mm, 2.3 µm)<br>Mobile Phase: Acetonitrile and 0.05% formic acid [40:60 v/v].<br>Animal: Male Sprague–Dawley rats (8–10 weeks old)<br>Internal Standard and Metabolite: BR-A-557<br>Blood Sample Collection Time: 0, 2, 5, 10, 15, 30 min, 1,2, 4, 8, 12, 24, 48 and 72 h. |

## 2.5 Discussion

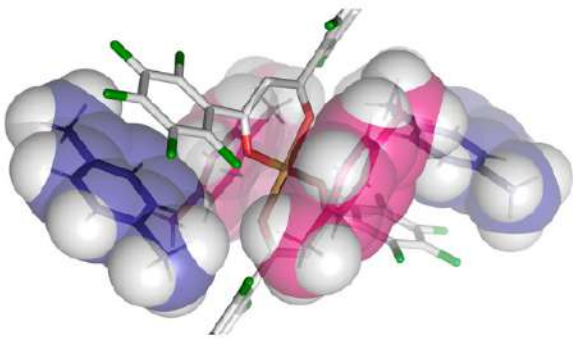
The literature review for FPT involved a comprehensive study of existing analytical and bioanalytical methods. This exploration served as a valuable foundation for both qualitative and quantitative assessments of FPT, its coformers, and the newly developed cocrystal, with necessary adjustments made to meet specific research needs. By assimilating knowledge from previous studies, the review not only facilitated a deeper understanding of analytical techniques but

also provided essential insights for the formulation and optimization of methods for FPT and its cocrystals.



# Chapter-3

# Aim and Objectives



# CHAPTER - 3

# Aim and Objectives

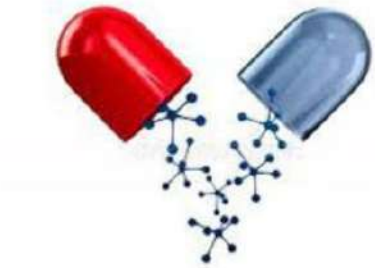
Fimasartan Potassium Trihydrate is an anti-hypertensive molecule belonging to BCS class II that is low solubility and high permeability. It possesses a low bioavailability of 18.6 %. This leads to higher doses with the potential for greater side effects.

## 3.1 Aim of the work

To design, synthesize and evaluate pharmaceutical cocrystal of Fimasartan Potassium Trihydrate.

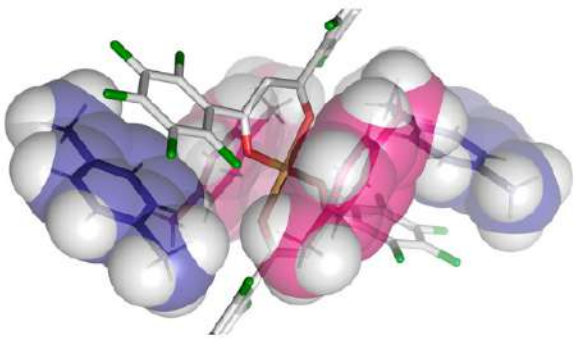
## 3.2 Objectives

- To investigate suitable coformers and method of preparation for cocrystal.
- To characterize the developed cocrystals by: Fourier-transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA), Powder X-ray Diffraction (PXRD), Single Crystal X-ray Diffraction (SCXRD), Dissolution Study, Solubility Study and Stability Study.
- To perform pharmacokinetic studies and to determine the % bioavailability.
- To prepare a suitable dosage form after preformulation studies and evaluation of the dosage form along with stability assessment.
- To establish an in-vitro-in-vivo correlation (IVIVC).



# Chapter-4

# Materials and Instruments



# CHAPTER - 4

# Materials and Instruments

Variety of instruments and materials were used throughout the research work for the preparation and analysis of cocrystals. The materials and instruments used in the formulation and pharmacokinetic studies are also listed below in tables 4.1 and 4.2.

## 4.1 List of Instruments

The instruments used throughout the experiments performed are listed in Table-4.1.

Table-4.1 List of Instruments

| Sr.No. | Instrument | Model no. | Manufacturer |
|---|---|---|---|
| 1. | Microbalance | XPE-26 | Mettler Toledo |
| 2. | Analytical Balance | XPE-205 | Mettler Toledo |
| 3. | Water Purification System | DQ 5 | Merck |
| 4. | Melting Point Apparatus | VMP-D | Veego, Mumbai |
| 5. | HPLC with PDA detector | Prominence-i Series Plus LC-2030C 3D PDA | Shimadzu |
| 6. | UV-Vis Spectrophotometer | Evolution 201 | Thermo Electron Scientific Instrument |
| 7. | FTIR | IR Spirit | Shimadzu |
| 8. | Refrigerated Centrifuge | Varsati T1000R | Esco Biotech |
| 9. | LC-MS/MS | 6545 XT (LC/Q-TOF) | Agilent |
| 10. | SEM | JSM-7600F | Elecmi |
| 11. | TEM | Transmission Electron Microscope 200KV | Thermo Fischer |
| 12. | PXRD | XR Dynamic 500 | Anton Paar |
| 13. | DSC | DSC 25 | TA Instruments |
| 14. | Particle Size Analyzer | Mastersizer 3000 | Malvern |
| 15. | Stability Chamber | GMP-SC90L | Kesar Controls |
| 16. | Supercritical Fluid Extractor | SFE-2000 | Jasco |
| 17. | pH meter | LMPH 10 | Labman |

## 4.2 List of Chemicals and Reagents

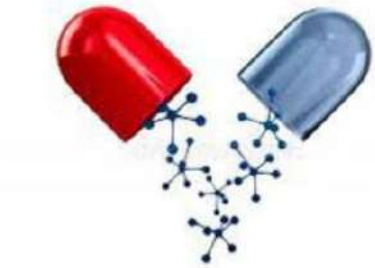
Fimasartan Potassium Trihydrate was procured from Kimia Biosciences Limited, Haryana, India. The lists of reagents used throughout the experiment are listed in Table-4.2 and the list of coformers used is stated in Table-4.3.

Table-4.2 List of Chemicals and Reagents

| Sr.No. | Reagents | Grade | Manufacturer |
|---|---|---|---|
| 1. | Methanol | HPLC | Merck Life Science Pvt. Ltd. |
| 2. | Acetonitrile | HPLC | Fisher Scientific |
| 3. | Methanol | AR grade | CDH Chemicals |
| 4. | Acetone | AR Grade | CDH Chemicals |
| 5. | EDTA | AR Grade | Lobal Chemie Pvt. Ltd. |
| 6. | Losartan | API | procured as gratis sample from Zydus Cadila, Ahmedabad, India |

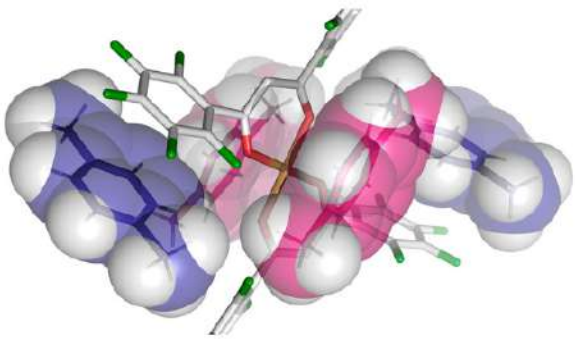
Table-4.3 List of Coformers

| Sr.No. | Coformer | Type | Grade | Source |
|---|---|---|---|---|
| 1. | Saccharine | Excipient | AR | CDH Fine Chemical |
| 2. | Succinic acid | API | AR | CDH Fine Chemical |
| 3. | Hydrochlorothiazide | API | procured as gratis sample from Fredun Pharmaceuticals Ltd, Thane, India. | |
| 4. | L – Proline | AA (NP-R) | procured as gratis sample from Clear Synth, Mumbai, India | |
| 5. | L – Valine | AA (NP-R) | | |
| 6. | L – Threonine | AA (P-R) | | |
| 7. | L – Cystine | AA (P-R) | | |
| 8. | L – Lysine | AA (Pos-R) | | |
| 9. | L – Glutamic acid | AA (Neg-R) | | |
| 10. | Glycine | AA (P-R) | | |
| AA: Amino Acid; NP-R: Non polar R group; P-R: Polar R-group; Pos-R: Positively charged R group; Neg-R: Negatively charged R group | | | | |



# Chapter-5

# Experimental Work



# CHAPTER - 5

# Experimental Work

The experimental work was planned with procurement of drug and it's authentication; preparation of cocrystals which included investigation and selection of coformers, techniques and stoichiometric ratio; physicochemical characterization of prepared cocrystals by various characterization techniques; pharmacokinetic studies; preformulation studies, dosage form preparation, evaluation and stability studies followed by In-vitro In-vivo Correlation (IVIVC). The experimental work is further divided into four chapters: Preparation and Characterization of cocrystals, Pharmacokinetics Study, Formulation Study and IVIVC Study.

## 5.1 Authentication of drug

The identification of the drug Fimasartan Potassium Trihydrate (FPT) was performed by melting point determination, IR Spectral determination and wavelength determination. An RP-HPLC method was also developed and validated which has been used throughout then work for quantification of FPT.

### *5.1.1 Melting Point Determination*

Melting point of FPT was determined using Veego melting point apparatus by open capillary method. The observed melting point along with the reported value is shown in Table-5.1.

Table-5.1 Melting Point Determination

| Reported Melting Point Range[58] | Observed Melting Point Range |
|---|---|
| 154-157 °C | 153-155 °C |

### *5.1.2 IR Spectrum Recording*

IR spectrum of FPT was recorded using FTIR, by mixing the sample uniformly with KBr. FTIR spectra of FPT was recorded by scanning it in the range of 200-4000 $cm^{-1}$. FTIR spectrum and its interpretation with standard values of functional group are shown in Fig-5.1 and Table-5.2 respectively.

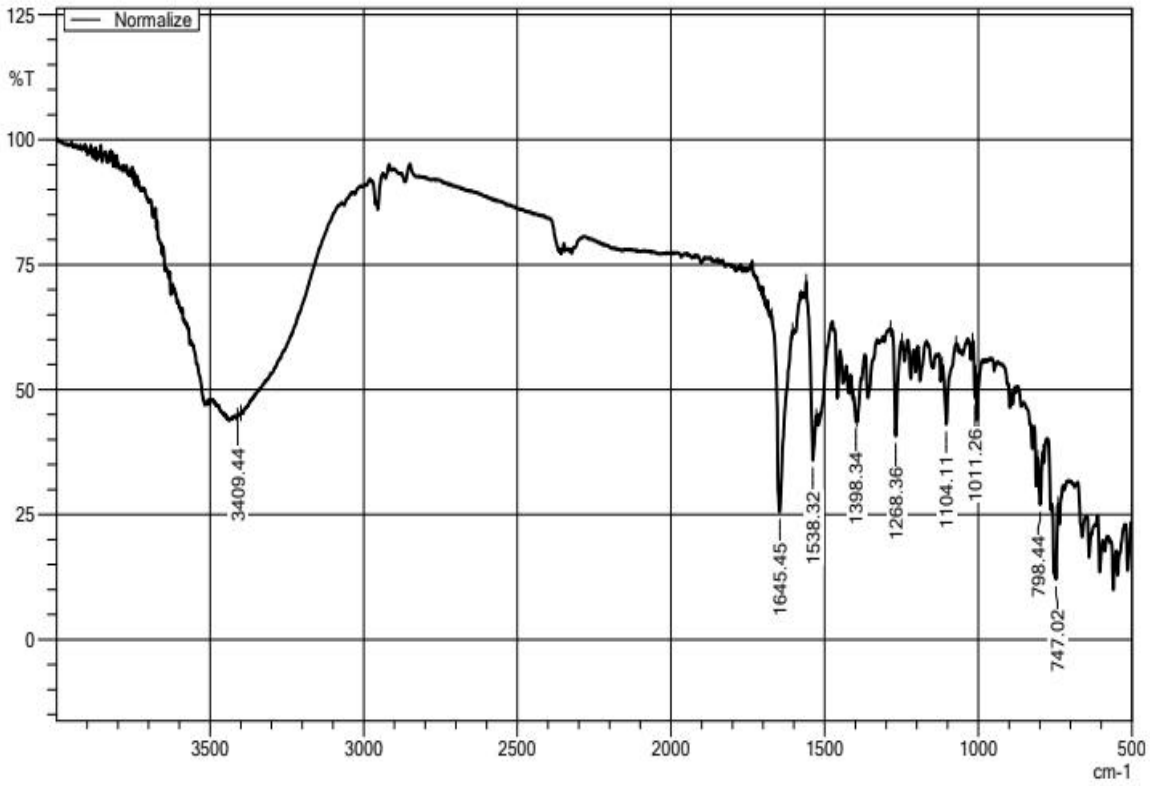



Figure-5.1 Recorded IR spectrum of FPT

Table-5.2 Interpretation of FTIR spectrum of FPT

| Functional Group | Standard wavenumber range ($cm^{-1}$) | Observed wavenumber ($cm^{-1}$) |
|---|---|---|
| N-H | 3440-3300 | 3409.44 |
| C=N | 2410 – 2320 | 2350.00 |
| C=O | 1700-1670 | 1645.45 |
| C-S | 700-550 | 640.00 |

### *5.1.3 Wavelength Determination*

The wavelength maximum of FPT was determined by U.V. visible spectrophotometer. The calibration curve was plotted in methanol throughout the range of 5 - 40 µg/mL with each increment of 5 µg. The samples were prepared from the working solution (100 µg/mL) obtained by the dilution of stock solution (1000 µg/mL) using water. The $\lambda_{max}$ obtained throughout the calibration range was 260nm. Fig-5.2 and Table-5.3 show the overlay spectrum and the calibration range of FPT respectively. The coefficient of regression was 0.9909 (Fig-5.3).

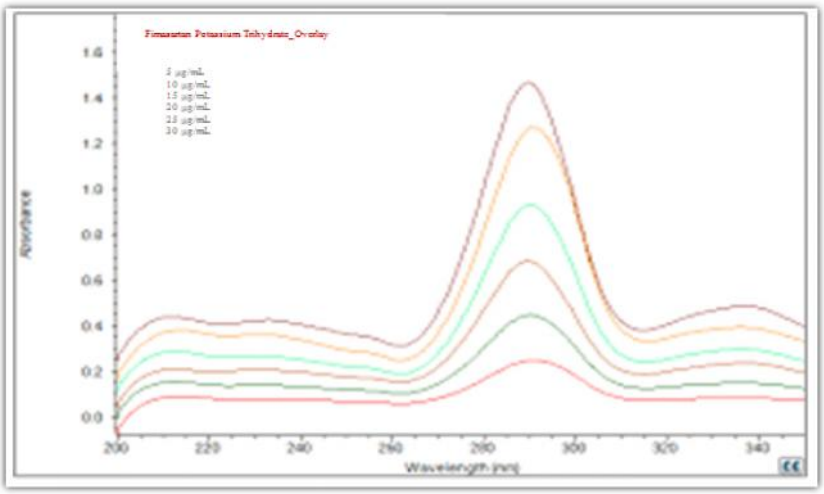


Figure-5.2 Overlay spectrum of U.V. visible spectroscopy of FPT

Table-5.3 Observation Table for U.V. visible spectroscopy study for FPT

| Concentration (µg/mL) | Absorbance (n-5) | % RSD |
|---|---|---|
| 5 | 0.09 | 1.09 |
| 10 | 0.15 | 1.75 |
| 15 | 0.35 | 1.63 |
| 20 | 0.57 | 0.98 |
| 25 | 0.76 | 1.37 |
| 30 | 0.94 | 1.83 |
| 35 | 1.10 | 1.21 |
| 40 | 1.37 | 0.50 |

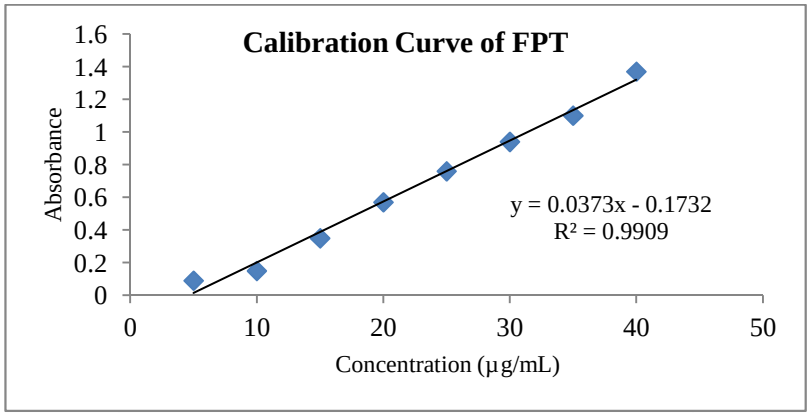


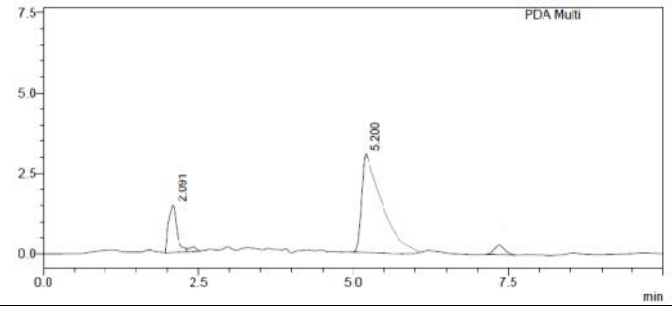
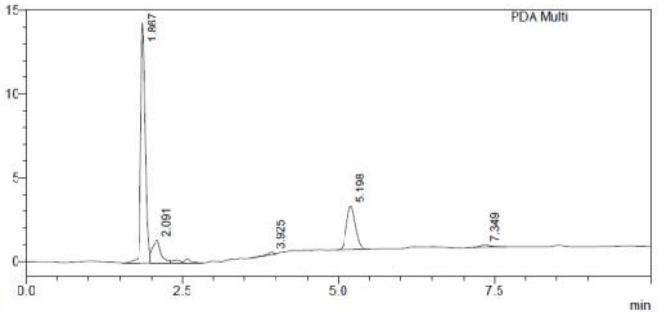
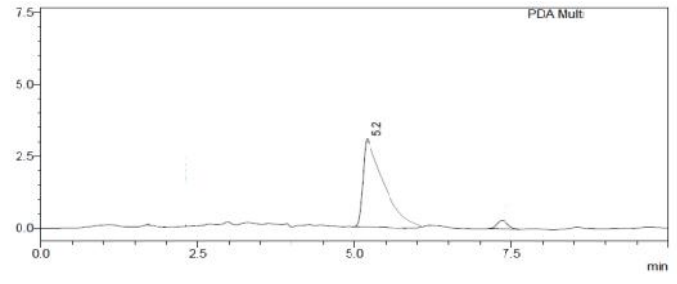
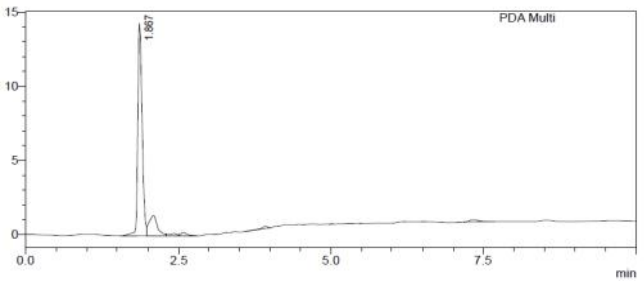

Figure-5.3 Calibration Curve of FPT

### *5.1.4 HPLC Method Development and Validation*

RP-HPLC method was developed for FPT by optimizing the mobile phase, flow rate and run time. A variety of mobile phase were tried and studied as shown in Table-5.4. The reported methods showed interference by the selected coformer and hence there was a need to develop a new method to fulfill the requirements of

the present work. The optimized chromatographic conditions are described in Table-5.5. The HPLC grade water is used throughout for the RP-HPLC analysis.

Table-5.4 Preliminary optimization trials of Mobile phase for analysis of FPT

| Sr. No. | Mobile Phase with variable Ratios / Flow Rate | Chromatogram | Observation |
|---|---|---|---|
| 1-3 | Phosphate Buffer pH-3: ACN (50:50 v/v 30:70 v/v 80:20 v/v) [1 mL/ min] |  | Dual peaks were observed with tailing more than 2.0. |
| 4-6 | Phosphate Buffer pH-3: Methanol (50:50 v/v 30:70 v/v 80:20 v/v) [1 mL/ min] |  | Multiple peaks were observed. |
| 7-10 | ACN: water (50:50 v/v 20:80 v/v 30:70 v/v 80:20 v/v) [1 mL/ min] |  | Tailing factor more than 2.00 was observed. |
| 11-13 | Methanol: water (50:50 v/v 30:70 v/v 80:20 v/v) [1 mL/ min] |  | Peak shape was good but elution was early at 1.86 min, and succinic acid showed up adjacent at 2.10 min |

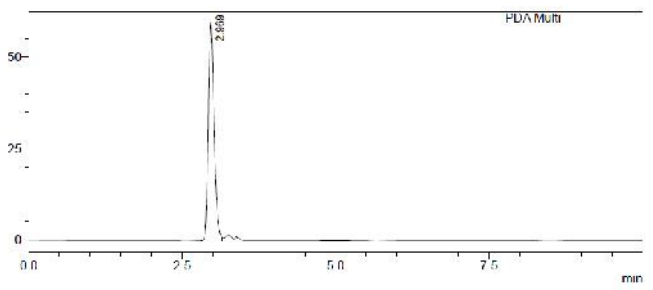
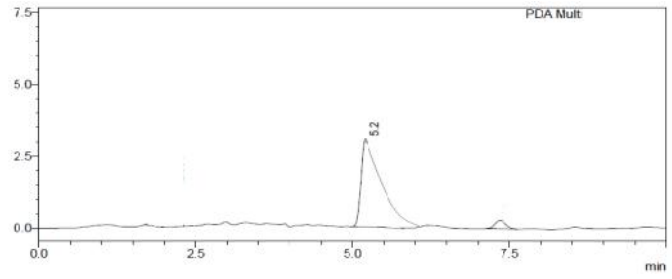
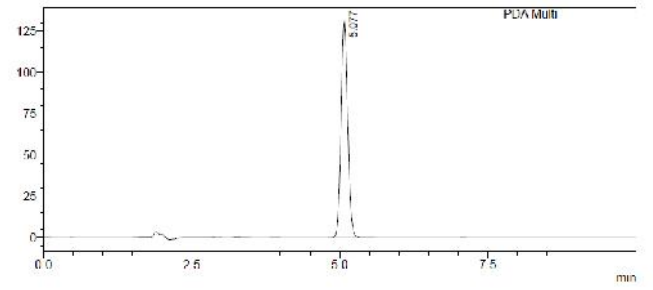
| | | | |
|---|---|---|---|
| 14-17 | Methanol: water (50:50 v/v 30:70 v/v 80:20 v/v 90:10 v/v) [0.8 mL/ min] |   | Good peak resolution at 2.96 min, but HCTZ and saccharine showed up between 2.5 to 3 min. |
| 18-21 | Methanol: water (80:20 v/v, 70:30 v/v, 20:80 v/v, 30:70 v/v) [0.8 mL/min] |   | Improper Peak Shape |
| 22 | Methanol: water (90:10 v/v) [0.8 mL/min] |   | Good peak shape at 5.07 min. |

Table-5.5 Optimized Chromatographic Conditions for RP-HPLC

| **Parameter** | **Conditions** |
|---|---|
| Mobile Phase | Methanol : Water (90:10 v/v) |
| Stationary Phase | RP-C18, 250 mm × 4.6mm, 5µ |
| Flow Rate | 0.8 mL/min |
| Injection volume | 10 µL |
| Detection Wavelength | 260 nm |
| Column Temperature | Ambient |
| Run Time | 10 min |

### *5.1.5 Validation of Developed Method*

The developed method was validated as per ICH Q2 guidelines that includes; linearity, precision, specificity, limit of detection, limit of quantification and recovery.

5.1.5.1 Linearity and Range

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity of response was assessed by analysis of five levels of

calibration curve in the range of 5-30 µg/mL (n=5). From the working standard solution (100 µg/mL), aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL were taken in 10 mL volumetric flask and diluted upto the mark with mobile phase. Mean peak area of each concentration was recorded (Table-5.6) and calibration curve was plotted that stated correlation co-efficient as 0.9934 (Fig-5.4).

Table-5.6 Observation Table for Linearity of FPT in RP-HPLC

| Concentration (µg/mL) | Mean Peak Area |
|---|---|
| 5 | 278750.33 |
| 10 | 527440.00 |
| 15 | 767726.33 |
| 20 | 980002.00 |
| 25 | 1149689.00 |
| 30 | 1326331.33 |

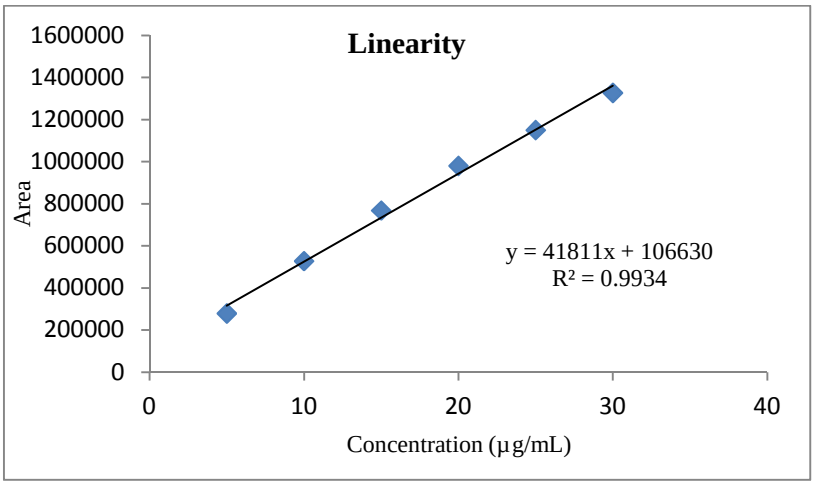



Figure-5.4 Linearity Study of FPT in RP-HPLC

5.1.5.2 Precision

5.1.5.2.1 Repeatability

Repeatability was performed by analyzing mid concentration (15 µg/mL) within the linearity range (n=6). The area was measured and % RSD was calculated which was 0.66 %. (Table-5.7)

Acceptance criteria: % RSD should not be more than 2.0 %.

Table-5.7 Data for Repeatability study of FPT

| Concentration | Peak Area (n-6) ± SD | % RSD |
|---|---|---|
| 15 µg/mL | 767726.33 ± 1328.85 | 0.66 |

5.1.5.2.2 Intermediate Precision

Precision was carried out by repeating 3 levels of calibration curve (10,15,20 µg/mL).

Interday Precision: The samples were injected at three consecutive days and % RSD calculated was found to be in the range of 0.57-1.72 %.

Intraday Precision: The samples were injected at different time interval in a day and analyzed to get % RSD. It was in the range of 0.07-1.25 % (Table-5.8).

Table-5.8 Data for Intra and Interday Precision of FPT

| Concentration (µg/mL) | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|
| | Area (n-3) | % RSD | Area (n-3) | % RSD |
| 10 | 527440.00 | 0.57 | 526991.10 | 0.07 |
| 15 | 767726.30 | 1.72 | 763318.10 | 1.00 |
| 20 | 980002.00 | 1.55 | 972615.00 | 5.1 |

5.1.5.3 Limit of Detection and Limit of Quantification

The lowest amount of the analyte in a sample which can be detected but not necessarily quantitated as an exact value is termed as LOD, while the lowest amount of analyte which can be quantitatively determined with suitable precision and accuracy is termed as LOQ. LOD and LOQ were calculated from the standard deviation of intercepts and mean slope of the calibration curves which was found to be 1.54 and 4.67 µg/mL respectively. The formula used are LOD = $(3.3\times\sigma)$ /S and LOQ = $(10\times\sigma)$ /S, where σ = Standard deviation of the Y intercept regression lines and S = Slope of the calibration curve equation.

5.1.5.4 Recovery

The accuracy of the method was determined by measuring the recovery of FPT using standard addition method. Known amounts of FPT were spiked at three levels to pre analyzed tablet sample. The quantity of formulation equivalent to 10 mg was transferred to four volumetric flasks. Standard FPT 8 mg,10 mg and 12 mg was spiked in second , third and fourth flasks respectively. All the flasks were filled with 80 % methanol and sonicated for 45 minutes and diluted upto the mark

with methanol. These solutions were filtered through Whatman filter paper individually. From each filtrate 1 mL was diluted to 10 mL with methanol. Each resulting solution was individually injected. The study was performed in triplicate. From the calibration curve, the amount of FPT recovered was calculated which was in between 98.97-99.86 %. (Table-5.9)

Table-5.9 Data for Recovery Studies of FPT

| Level (%) | Quantity Taken (mg) | Quantity Added (mg) | Quantity recovered (mg) (n-3) | Mean | SD | % RSD | % Recovery |
|---|---|---|---|---|---|---|---|
| 0 | 10 | - | 9.88 | 9.89 | 0.17 | 0.37 | - |
| 80 | 10 | 8 | 7.90 | 7.90 | 0.04 | 0.51 | 99.83 |
| 100 | 10 | 10 | 9.89 | 9.89 | 0.18 | 1.84 | 98.97 |
| 120 | 10 | 12 | 11.98 | 11.98 | 0.04 | 0.35 | 99.86 |

5.1.5.5 Robustness

Robustness of the method was assessed by the effect of variation in parameters like flow rate, column oven temperature, detection wavelength and composition of the mobile phase for HPLC studies.% RSD of peak area was found to be 0.09 % to 0.11 %; hence it indicates that the method was robust (Table-5.10).

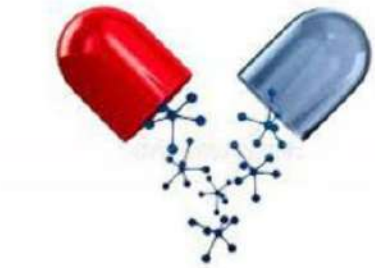
Table-5.10 Data for Robustness Studies of FPT

| Parameter | Change in Parameter | Peak Area | Mean (n=3) | %RSD |
|---|---|---|---|---|
| Flow Rate (mL/ min) | 0.8 | 536985 | 536451.7 | 0.09 |
| | 1 | 536439 | | |
| | 1.2 | 535931 | | |
| Wavelength (nm) | 258 | 536197 | 535590.7 | 0.23 |
| | 260 | 536439 | | |
| | 262 | 534136 | | |
| Mobile phase (v/v) | 100:00 | 537625 | 537123.3 | 0.11 |
| | 90:10 | 536439 | | |
| | 80:20 | 537551 | | |

**5.2 Discussion**

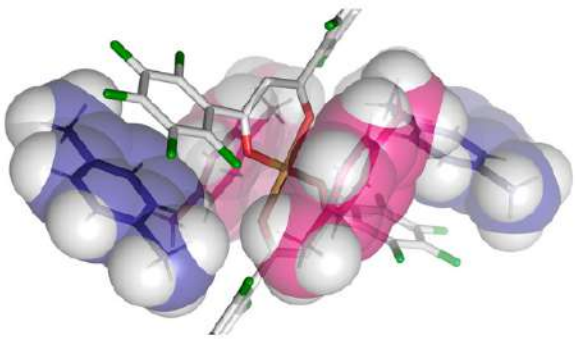
The experimental work described in this chapter was conducted by focusing on the API for the project that is FPT. Authentication of FPT was performed by melting point, FTIR studies and wavelength determination. These studies proved that the FPT API received as a test sample for rest of the experiments was similar

to that of standard FPT. An accurate, selective, sensitive, robust and precise RP-HPLC method was developed for routine analysis. It was validated as per ICH Q2B guidelines and all the validation parameters were well within the stated limits.



# Chapter-6

# Preparation and Characterization of Cocrystals



# CHAPTER - 6

# Preparation and Characterization of Cocrystals

The preparation of cocrystals initiates with the investigation into the tendency to form cocrystal. Various in-silico and computational studies are undertaken to determine the cocrystal formation. In-silico techniques help in giving valuable insights and predictions to streamline the following experimental work. Numerous methods are employed for cocrystal preparation, each exhibiting varying yields and success rates in cocrystal formation. The evaluation and identification of cocrystals is accomplished through diverse characterization techniques, including spectrophotometric, analytical, microscopic and diffraction methods. These techniques enable the differentiation and recognition of cocrystals in comparison to the Active Pharmaceutical Ingredient (API), coformer, and Physical Mixture (PM).

There are numerous factors that influence the cocrystal formation, and fine-tuning these variables can lead to the development of optimized cocrystals.

*Molecular Compatibility*: The ability of the API and coformer molecules to interact and form stable hydrogen bonds or other intermolecular forces is crucial.

*Stoichiometry*: The ratio of API to coformer plays a significant role in determining the formation and stability of the cocrystal.[3]

*Polymorphism*: Different polymorphic forms of the API and coformer can impact the cocrystallization process and the resulting cocrystal's properties.

*Solubility*: The solubility of the API and coformer in the selected solvent can influence cocrystal formation, as well as the choice of solvent for crystallization.[4]

*Temperature and Pressure*: Conditions such as temperature and pressure during the cocrystallization process can affect the formation and stability of the cocrystal.

*pH*: The pH of the solution can influence the ionization state of the API and coformer, thus affecting their ability to interact and form cocrystals.

*Presence of Impurities*: Impurities can interfere with the cocrystallization process, affecting the purity and yield of the cocrystals.

*Choice of Solvent*: The solvent used for crystallization must adequately dissolve both the API and the coformer and promote the formation of a stable cocrystal structure.[5]

## 6.1 Computational Studies

Computational studies play a pivotal role in predicting the understanding of cocrystal formation. Through molecular modeling and simulations, these studies provide insights into the possible intermolecular interactions between the API and the coformer. By predicting potential cocrystal structures and analyzing their stability, computational methods assist in selecting promising candidates for experimental synthesis. Moreover, they offer a cost-effective way to explore various bonding patterns and identify hydrogen bonding interactions, which are crucial for cocrystal formation.

The inter and intramolecular bonding between FPT and various coformers were studied with help of various predictability parameters and software.

### 6.1.1 In-silico cocrystal screening

The in-silico cocrystal screening was studied by downloading the 3-D Structures from Cambridge Structural Database (with R-factor less than 5, to avoid any disorder in the structure). Material Studio was used for optimizing the geometry of the structure (crystal lattice, cell parameters). The optimized structures were imported in Mercury Software (MOPAC Version - 3.10) and the parameters were calculated. The data depicts the formation of intermolecular and intramolecular H-bond and also shows the difference in the heat of formation and ionization potential between FPT and the coformers (Table-6.1).

Table-6.1 In-silico cocrystal screening

| Rank | Chemical Name | Materials Studio 2 | Parameters studied using Mercury Version 3.10 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | No of molecules in crystal lattice | Inter Mol. H-bond | Intra Mol. H-bond | Heat of formation | Diff in heat of formation | Final Heat of formation | Ionization Potential |
| API | FPT | 4 | 2 | 2 | 3209.46 | 0 | -52.13 | 8.55 |
| 1 | Saccharine | 8 | 0 | 3 | 1556.53 | 1652.93 | 350.01 | 9.21 |
| 2 | Succinic acid | 20 | - | - | 3276.73 | -67.27 | -136.93 | 8.94 |
| 3 | HCTZ | 4 | 2 | 1 | 854.63 | 2354.83 | 89.72 | 10.38 |
| 4 | L-Proline | 4 | 1 | 1 | 992.69 | 2216.77 | 497.93 | 8.16 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | L-Valine | 4 | 1 | 6 | 668.7 | 2540.76 | 222.83 | 8.37 |
| 6 | L-Threonine | 4 | 0 | 0 | 746.18 | 2411.65 | 955.04 | 6.25 |
| 7 | L-Cystine | 4 | 1 | 7 | 28.37 | 3181.09 | -242.09 | 8.91 |
| 8 | L-Lysine | 2 | 0 | 5 | -5.78 | 3215.24 | 21.3 | 9.27 |
| 9 | L-Glutamic acid | 4 | 0 | 4 | -165.32 | 3836 | -620.57 | 10.57 |
| 10 | Glycine | 4 | 2 | 6 | 20.22 | 3189.23 | -289.84 | 7.94 |

6.1.2 pKa rule

The formation of salts or cocrystals can be predicted by determining the ΔpKa using the formula: ΔpKa = [pKa (FPT) – pKa (Coformer)]. It is generally accepted that proton transfer will occur from an acid to a base if the difference in the pKa values is greater than 3, which will lead to salt formation. If the ΔpKa value is less than or near to 0, the probability of cocrystal formation is much higher. On the other hand, if the ΔpKa value is between 0-3 the molecule has the tendency to behave as both salt and cocrystal.[32]

Table-6.2 ΔpKa study of Coformer

| Sr. No. | Chemical Name | H-bond acceptor | H-bond donor | pka acid | pka base | pka difference |
|---|---|---|---|---|---|---|
| API | FPT | 10.00 | 0.00 | 4.23 | 9.72 | 0.00 |
| 1 | Saccharine | 4.00 | 0.00 | 2.32 | 8.6 | 0.02 |
| 2 | Succinic acid | 2.00 | 0.00 | 4.25 | 5.64 | 2.29 |
| 3 | HCTZ | 5.00 | 3.00 | 4.17 | 9.35 | -0.06 |
| 4 | L-Proline | 3.00 | 2.00 | 4.74 | 10.62 | -0.51 |
| 5 | L-Valine | 3.00 | 2.00 | 2.90 | 9.74 | 1.94 |
| 6 | L-Threonine | 4.00 | 3.00 | 2.09 | 9.10 | 2.14 |
| 7 | L-Cystine | 8.00 | 4.00 | 1.92 | 10.70 | 2.31 |
| 8 | L-Lysine | 4.00 | 3.00 | 2.16 | 9.06 | 2.07 |
| 9 | L-Glutamic acid | 5.00 | 3.00 | 2.10 | 9.47 | 2.13 |
| 10 | Glycine | 3.00 | 2.00 | 2.29 | 9.74 | 1.84 |

The above listed computational studies provided an insight into the likely coformers to be used for cocrystal formation of FPT. The subsequent computational studies namely cocrystal structure analysis, Hirshfeld surface area and surface electrostatic potential were done only for the optimized cocrystal formed using L-Proline as the coformer in the ratio 1:2 (FPT: L-proline) with FPT.

6.1.3 Cocrystal Structure Analysis

The coformer was selected from the range after the completion of basic trials with each technique. The selected coformer L-proline (LP) showed maximum cocrystal yield during the preliminary trials. Hence, cocrystal structure analysis of the developed cocrystals of FPT-LP was studied that showed the crystal system as Monoclinic (Table-6.3).The FPT-LP showed a 'sandwich-like' trimer formation (Fig-6.1); where the two white lines depict LP while one green line depicts FPT. The numbers in bracket in the Data column (Table-6.3) refers to number of times.

Table-6.3 Cocrystal Structure Analysis of FPT-LP

| Parameters | Data |
|---|---|
| Crystal size | 0.79, 0.63, 0.32 |
| Temperature (ºC) | 293 |
| Crystal system | Monoclinic |
| Space group | P21/c |
| A | 15.771 (11) |
| B | 7.46 (4) |
| C | 16.570 (11) |
| Alpha | 90 |
| Beta | 115.985 (13) |
| Gama | 80 |
| Z | 4 |
| Volume (A cube) | 1749.20 (19) |
| Calc density (g.cm-3) | 1.41 |
| Absorption coefficient (mm-1) | 1.90 |
| Goodness to fit on F2 | 1.06 |
| Final R | 0.05 |
| Completeness | 0.96 |

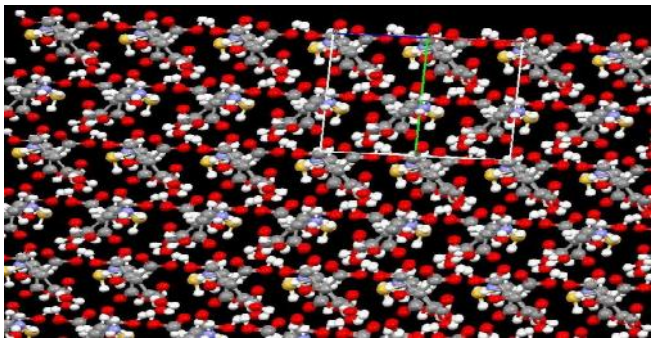


Figure-6.1 Sandwich like Trimer of FPT-LP

6.1.4 Hirshfeld Surface Analysis (HSA)

Hirshfeld Surface Analysis (HSA) serves as a tool for both quantifying and visually representing the nature and strength of intermolecular interactions within a crystal lattice. This analysis involves color-coding the surface to denote various types of interactions, including hydrogen bonding, van der Waals interactions, and other close contacts. By using electron density data, the Hirshfeld surface is calculated and depicted with distinct colors corresponding to different interaction types. The CrystalExplorer software is employed to conduct HSA. Fig 6.2 (a) depicts the binding site while Fig-6.2 (b) and (c) provide a visual representation of the extent of bonding at each specific site.

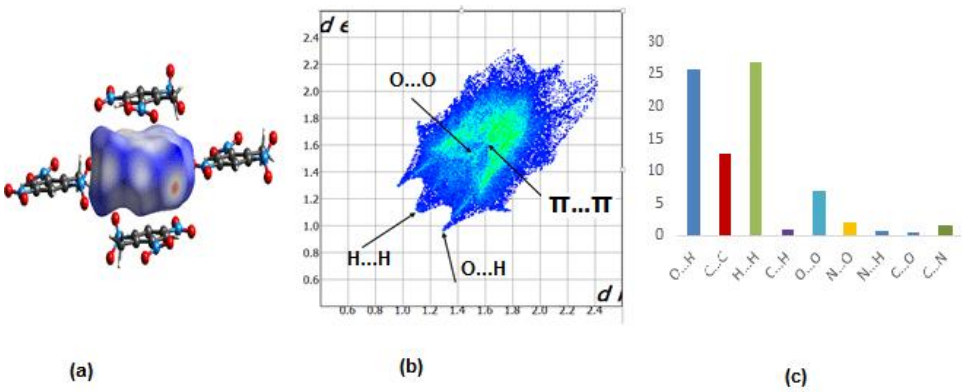



Figure-6.2 Hirshfield Surface Analysis of FPT-LP
(a) Binding site (b) Two dimensional fingerprint (c) Contact contribution ratio

6.1.5 Surface Electrostatic Potential (SEP)

SEP illustrates charge distribution three dimensionally. Mercury software version 3.10 is used to determine this study. The main site of interaction in the cocrystal first occurs pair wise in the primary minima and maxima of the SEP on the surface, followed by the secondary ones. The plane of FPT gets rotated because of repulsion between the FPT (global minima site) and coformer (secondary minima

site). Table-6.4 shows the target sites with global maxima and global minima value of FPT and coformer in correspondence to the functional group as shown in Fig-6.3.

Table-6.4 Surface Electrostatic Potential of FPT-LP

| Name | Target sites | Global maxima (Kcal/mol) | Global minima (Kcal/mol) | Functional group |
|---|---|---|---|---|
| FPT | Local | +59.78 | +59.35 | -OH group |
| | Secondary | -31.01 | -31.56 | Carbonyl group |
| L-proline | Local | +52.65 | -79.99 | Sulphur ion |
| | Secondary | +41.12 | -42.10 | Amine group |

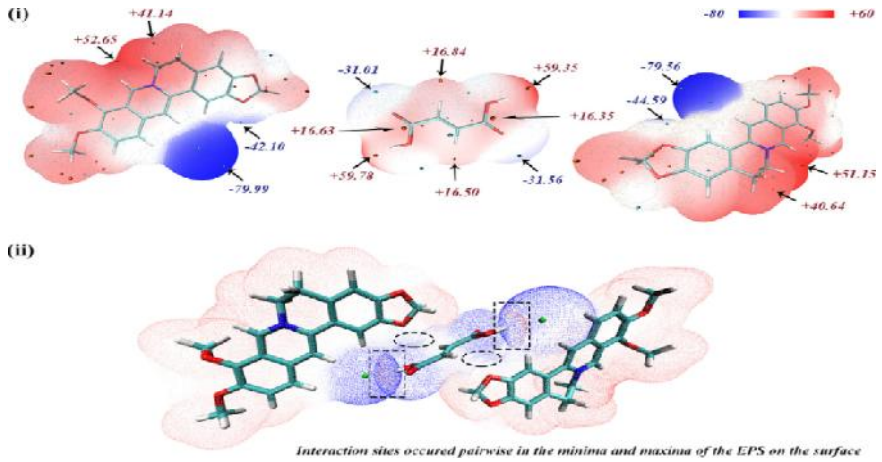



Figure-6.3 Surface Electrostatic Potential of FPT-LP
(i) Charged maxima sites (ii) Interaction sites

## 6.2 Techniques for Cocrystal Formation

A number of techniques are available for the preparation of cocrystals. The various techniques attempted for the formation of FPT cocrystals are Solvent Evaporation Technique, Cooling Method, Dry Grinding Method, Wet Grinding Method, Centrifugation Technique, Freezing Technique and Supercritical Fluid Extraction Technique. During the trial and error of the above techniques four samples were studied for each variable; namely drug and coformer individually, Physical Mixture (PM) and Treated sample (yield/ cocrystal). The list of all the coformers used throughout the trials in various stoichiometric ratios with FPT is stated in Table-6.5.

6.2.1 Solvent Evaporation Technique

The solvent evaporation technique is a common method used in the preparation of

cocrystals. This technique involved the dissolution of the components (API and coformer) in a common solvent, followed by controlled evaporation of the solvent to induce cocrystal formation. FPT and different coformers were studied with variations in solvent, solvent volume, stoichiometric ratio of FPT: coformer, temperature and time of evaporation (Table-6.5). FPT-saccharine, FPT-hydrochlorothiazide and FPT-L-proline showed cocrystal formation by this technique. FPT-L-Proline showed needle shaped crystals with the maximum yield in stoichiometric ratio 1:2 as shown in Fig-6.4(a).

6.2.2 Cooling Method

The cooling method involved the controlled cooling of a solution by mixing the API and coformer in a specific stoichiometric ratio in a suitable common solvent. This technique was explored with variations in solvent, stoichiometric ratio and the cooling time (h) as showed in Table-6.5. The solvent selection plays a vital role as both the components should be readily soluble in the selected solvent. This leads to appropriate cooling process.

6.2.3 Dry Grinding Method

The API and coformer were weighed in the desired stoichiometric ratio and mixed in a mortar with a pestle. The grinding process was commenced in a uniform direction at room temperature or under controlled conditions, depending on the properties of the API and coformer. The selection of precise ratio of API and coformer plays a crucial role for the formation of desired cocrystals. The trituration time (min), stoichiometric ratio of FPT: coformer was varied as shown in Table-6.5.

6.2.4 Wet Grinding Method

Wet grinding method is similar to dry grinding method (6.2.3), with the addition of a suitable solvent. The API and coformer were admixed with the suitable solvent. The variables like solvents, stoichiometric ratio, mixing time (min), solvent volume are stated in Table-6.5.

6.2.5 Centrifugation Technique

FPT and coformer were dissolved in the suitable solvent in varied stoichiometric ratio of FPT: coformer and centrifuged. The selection of solvent plays an important role as both the API and coformer should be easily soluble in the selected solvent, to get the formation of cocrystal. After the centrifugation, the solids from the

solution were filtered and dried to measure the yield. The variations in stoichiometric ratio, centrifugation time, speed and solvent is stated in Table-6.5

6.2.6 Freezing Technique

The solution was freezed after dissolving the appropriate proportions of API and coformer in a suitable solvent. The freezing technique involves the selection of the solvent in accordance to its freezing point to commence the cocrystal formation process. .

6.2.7 Supercritical Fluid Extraction Technique (SFE)

The SFE technique was used as a scale-up method for solvent evaporation technique. This technique was applied to only L-proline, with its definite stoichiometric ratio as it showed maximum cocrystal yield during solvent evaporation technique.

FPT and L-Proline were weighed in the stoichiometric ratio of 1:2 and mixed thoroughly. The mixture was placed in the sample compartment on cotton wool. Methanol and distilled water (5:95 v/v) was selected as the mobile phase for the extraction. Supercritical $CO_2$ was passed at 70°C and pressure 80.0 MPa. The extracted samples were collected in the collection tube. The solution from the collection tube was dried at room temperature to get the solid cocrystals (Fig-6.5). These are the optimized conditions for the extraction of the cocrystals. The variables undertaken during the preliminary trials of SFE include temperature variations of 50 and 90 °C and pressure 100 MPa. The cocrystal formation was observed as shown in Fig-6.4 (b) with more yield and in a time efficient manner.

(a) (b)

Figure-6.4 Cocrystals of FPT-LP
(a) Solvent Evaporation Technique (b) SFE Technique

Table-6.5 Details of Variables for the Techniques for Cocrystal Formation

| Sr. No | Parameter | Variables |
|---|---|---|
| **Coformer** | | L-Proline, Saccharine, Succinic acid, Hydrochlorothiazide, L-Valine, L-Threonine, L-Cystine, L-Lysine, L-Glutamic acid, Glycine |
| 1. | **Technique** | **Solvent Evaporation Technique** |
| | Solvent | Distilled water, methanol, IPA, distilled water: methanol distilled water: IPA, IPA: methanol, distilled water: methanol: IP (50:50, 70:30, 80:20, 30:70, 20:80) |
| | Solvent volume (mL) | 5,10,15,20,15,30,45,60,80 |
| | Water-bath Temperature (°C) | 60,70,85,90,125 |
| | Heating Time (min) | 10,15,20,25,30,40,50,60 |
| | Stoichiometric Ratio FPT: Coformer (w/w) | 1:1, 1:2, 2:1, 2:2 |
| 2. | **Technique** | **Cooling Method** |
| | Solvent | Distilled water, methanol, distilled water: methanol (50:50, 70:30, 80:20, 30:70, 20:80) |
| | Solvent volume (mL) | 5,10,15,20,25 |
| | Cooling Temp (°C) | 20, 5 |
| | Cooling Time (h) | 1,3,5,8,12,24,48 |
| | Stoichiometric Ratio (FPT: Coformer) (w/w) | 1:1, 1:2, 2:1, 2:2 |
| 3. | **Technique** | **Dry Grinding Method** |
| | Trituration Time (min) | 10,20,30 |
| | Stoichiometric Ratio (FPT: Coformer) (w/w) | 1:1, 1:2, 2:1, 2:2 |
| 4. | **Technique** | **Wet Grinding Method** |
| | Solvent | Distilled water, methanol, distilled water: methanol (50:50, 70:30, 80:20, 30:70, 20:80) |
| | Solvent volume (mL) | 5,10,15,20,25 |
| | Trituration Time (min) | 10,20,30 |
| | Stoichiometric Ratio (FPT: Coformer) (w/w) | 1:1, 1:2, 2:1, 2:2 |
| 5. | **Technique** | **Centrifugation Technique** |
| | Solvent | Distilled water, methanol, IPA, distilled water: methanol, Distilled water: IPA (50:50, 70:30, 80:20, 30:70, 20:80) |
| | Solvent volume (mL) | 5,10,15,20,25,50 |

| | | |
|---|---|---|
| | Centrifugation Speed (rpm) | 3000,4000,5000 |
| | Centrifugation Time (min) | 15,20,25,30 |
| | Stoichiometric Ratio (FPT: Coformer) (w/w) | 1:1, 1:2, 2:1, 2:2 |
| 6. | **Technique** | **Freezing Technique** |
| | Solvent | Distilled water |
| | Solvent volume (mL) | 10,20,50 |
| | Cooling Temp (°C) | -20, -80 |
| | Cooling Time (h) | 5,10,12,24,48 |
| | Stoichiometric Ratio (FPT: Coformer) (w/w) | 1:1, 1:2, 2:1, 2:2 |

### 6.3 Optimization of Coformer and Stoichiometric Ratio

The above techniques helped in identifying the coformers with the tendency to form cocrystals. These techniques were studied for all the selected coformers and varied stoichiometric ratio.

*Importance of coformer selection*: The selection of coformer is of profound importance as the physicochemical properties of the resulting cocrystal depend on the coformer. The coformer, chosen judiciously, plays a pivotal role in determining the stability, solubility, and bioavailability of the cocrystal. The regulatory compliance of cocrystal-based pharmaceuticals also hinges on a comprehensive understanding of coformer interactions and the resultant impact on product safety and efficacy.

*L-Proline as a coformer*: L-proline is the only amino acid with a five-member ring structure, the pyrrolidine ring that makes the structure rather rigid. In cocrystal formation, this stiffness is an advantage over the more flexible co-formers as it contributes to its stabilization ability. It is considered as an excellent candidate in cocrystal formation because it forms α-ammonium carboxylate zwitterions, which support atomic interactions. L-Proline has a reasonably broad zwitterion pH range of 1.8 to 10.63. The addition of L-proline as a coformer, leads to formation of ‘sandwich-like’ trimer in the cocrystal structure. L-proline is the most soluble amino acid and can dissolve hydrophobic molecules with a hydrotropic effect. Amino acids tend to form head-to-tail charge-assisted hydrogen-bonded chains during cocrystal formation, thereby increasing the percentage of strong interactions

in the cocrystal structure. L-proline exhibits the highest capacity to construct cocrystal and modulates the pharmaceutical performance due to its rigid zwitterionic structure, solubility and hydrotropic activity.

*Importance of stoichiometric ratio selection*: The stoichiometric ratio selection in cocrystal formation is a crucial parameter that profoundly influences the composition and properties of the resulting cocrystal. The ratio in which the coformer and API combine plays a pivotal role in defining the structure and performance of the cocrystal. The right stoichiometric balance is essential for maintaining the desired crystalline structure, as deviations from the ideal ratio can lead to the formation of impurities or amorphous phases. In pharmaceutical applications, precise stoichiometric control is paramount for optimizing drug formulation, affecting factors such as solubility, stability, and bioavailability. Moreover, the stoichiometric ratio influences the thermodynamic stability of the cocrystal, with some ratios exhibiting greater stability than others. The choice of stoichiometry also impacts the crystallization process and can affect the ease of manufacturing and reproducibility of cocrystals on an industrial scale.

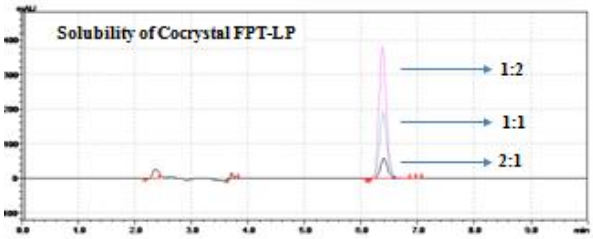
### 6.3.1 Solubility Studies

Solubility studies are crucial, as it provides essential insights into behavior of the drug in different environments, aiding in the formulation and development and designing of the chemical processes. The solubility of a compound determines its bioavailability, impacting the effectiveness of drugs.

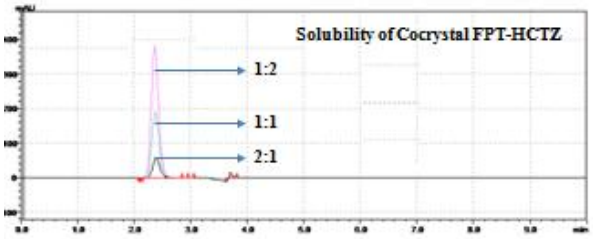
The solubility studies were conducted on promising cocrystal with coformers L-proline, HCTZ and saccharine. Different molar stoichiometric ratios like 1:1, 1:2, 2:1 and 2:2 were studied for promising conformers.

10 mg of FPT, PM and cocrystals were added in 3 different volumetric flasks containing 5 mL distilled water and shaken for 10 min on shaker. Distilled water was added upto the mark of the volumetric flask (10 mL) and shaken for further 10 min. The resulting solution was filtered using Whatman filter paper (1; 110 mm). The filtrate was analyzed with the developed RP-HPLC method (Refer: 5.1.4). The studies were conducted on various solvents and mixture of solvents like water and methanol. The solubility study was conducted for 24 hours for API, coformers, PM and cocrystals. The promising cocrystals were analyzed by RP-HPLC method for each stoichiometric ratio (Fig-6.5). This study focused on identifying the stoichiometric ratio with varied outputs like percentage yield and solubility.

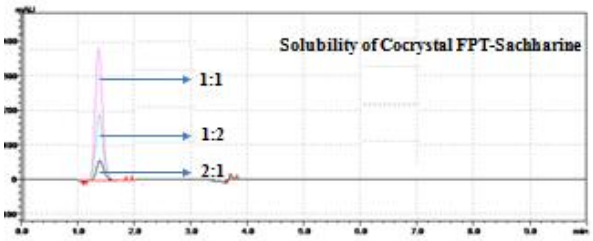
Inference: FPT-LP cocrystal showed maximum solubility with an increase by 8 folds, while FPT-HCTZ and FPT-Saccharine showed 4 and 3 folds increase respectively. The stoichiometric ratio of FPT-LP 1:2 (250 mg/g) showed maximum solubility and percentage yield in comparison to the ratios 1:1 (143 mg/g), 2:1 (77mg/g) and 2:2 (71 mg/g). The studies on PM, FPT and coformer did not show any significant increase in solubility.




| | |
|---|---|
| Yield (%) | **75.15** |
| Increase in solubility (folds) | **8** |
| Solvent for preparation (v/v) | **Methanol: water (20:80)** |




| | |
|---|---|
| Yield (%) | 69.31 |
| Increase in solubility (folds) | 4 |
| Solvent for preparation (v/v) | Methanol: water (50:50) |




| | |
|---|---|
| Yield (%) | 61.51 |
| Increase in solubility (folds) | 3 |
| Solvent for preparation (v/v) | Methanol |

Figure-6.5 Solubility Studies of FPT-LP and Optimization of Stoichiometric Ratio

## 6.4 Characterization of developed cocrystals

The characterization of developed cocrystals is a pivotal aspect of pharmaceutical research. There are various analytical techniques such as PXRD, SCXRD, DSC, TEM, SEM and FTIR to determine the structural, thermal and molecular attributes of the cocrystals. This multifaceted approach confirms the identity, stability, and potential for enhanced properties of the cocrystals. The preliminary characterization to confirm the formation of cocrystals was concluded by FTIR and DSC. The other techniques for characterization like SEM, TEM, PXRD, SCXRD, Phase transformation and solubility studies were performed for the optimized FPT-LP (1:2) cocrystal.

6.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a technique that analyzes the interaction between infrared light and a sample to identify functional groups and chemical bonds, providing insights into the composition of a material. It produces a spectrum representing the absorption of infrared radiation by the sample. In ATR-FTIR, infrared radiation penetrates the sample, and the ATR crystal reflects it back, generating an absorbance spectrum that reveals molecular information about the sample's composition and structure.

FPT-LP cocrystal was directly placed on the ATR crystal. The sample was pressed against the crystal to maximize contact, enhancing the signal strength. The recorded spectrum is depicted in Fig-6.8. The obtained spectrum was compared with the spectra of FPT, coformer and PM recorded in a similar manner under the same conditions. (Fig-6.6)

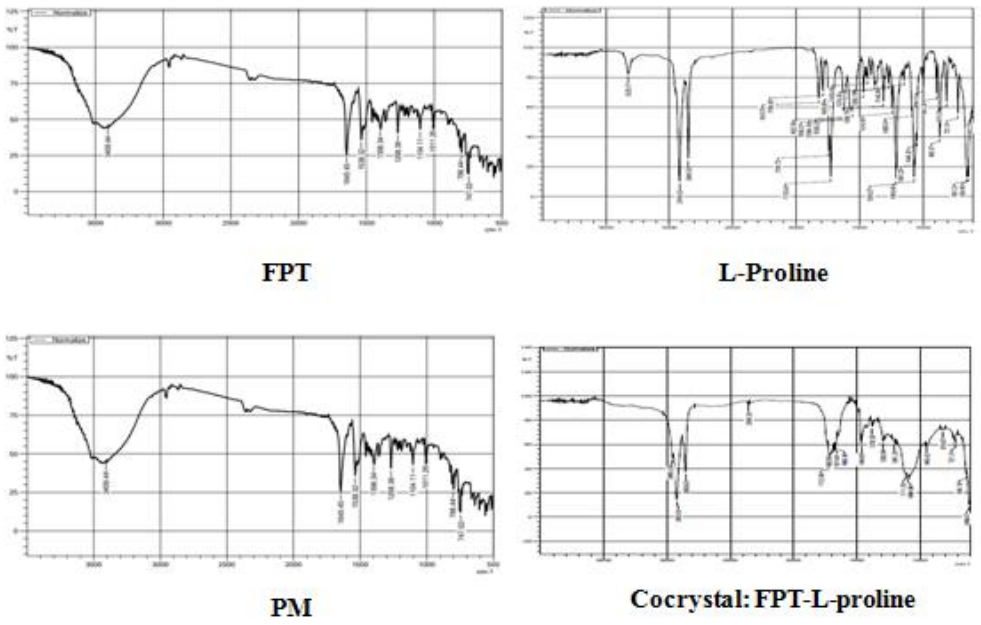



Figure-6.6 FTIR Studies of FPT, LP, PM and FPT-LP

6.4.2 Scanning Electron Microscope (SEM)

A SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample.

Sample preparation for SEM involved fixing and dehydrating the sample, followed by coating with a thin layer of conductive material such as gold or palladium. The sample was mounted onto conductive stubs using adhesive tapes, for stability and effective electrical conductivity of the sample during analysis. It was then carefully

loaded into the SEM chamber, and imaging parameters were adjusted. Detection was achieved by capturing secondary or backscattered electrons, providing detailed images for comprehensive analysis and characterization of the sample's surface morphology at high magnifications. The SEM image of FPT, LP, PM and FPT-LP is shown in Fig 6.7 a., b, c and d. respectively. All images were captured in 1000x magnification.

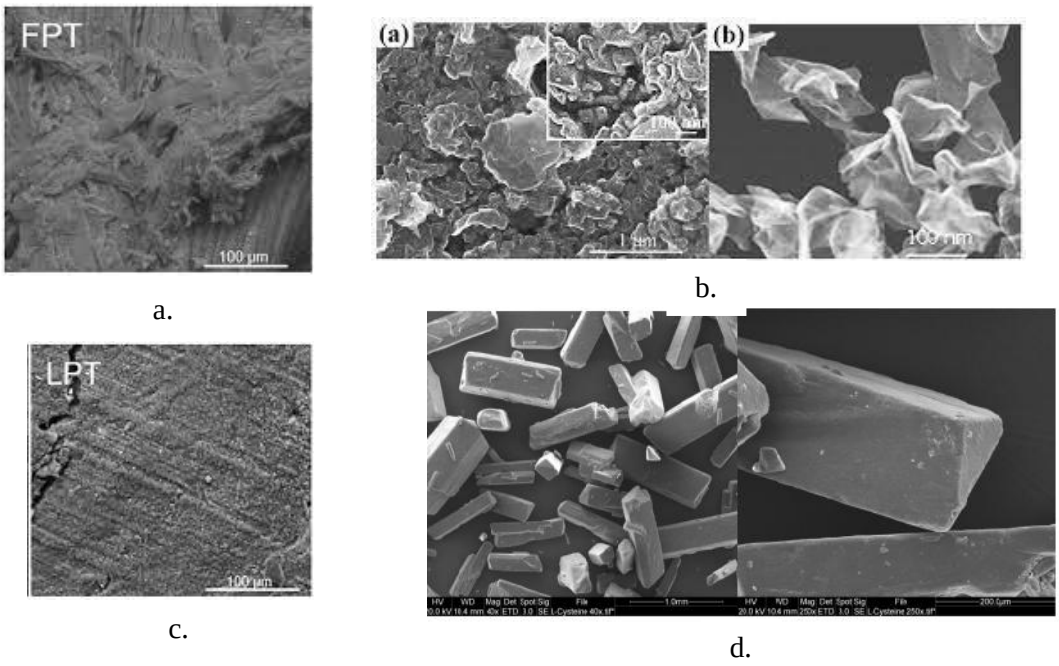



Figure-6.7 SEM studies of a. FPT b. LP c. PM d. FPT-LP

6.4.3 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through a sample to form an image. This enables to capture the detailing of the sample like a single column of atoms. TEM samples were prepared by fixing and dehydrating the cocrystal, followed by embedding it in resin for thin sectioning. Thin sections were cut using an ultramicrotome and transferred onto TEM grids to support the sample and facilitate electron transmission. This was later loaded into the TEM column and subjected to a high-energy electron beam, where the detectors capture transmitted electrons to produce high-resolution images for detailed analysis of internal structures at the nanoscale. The TEM image of FPT, LP and FPT-LP is shown in Fig-6.8 a., b. and c respectively.

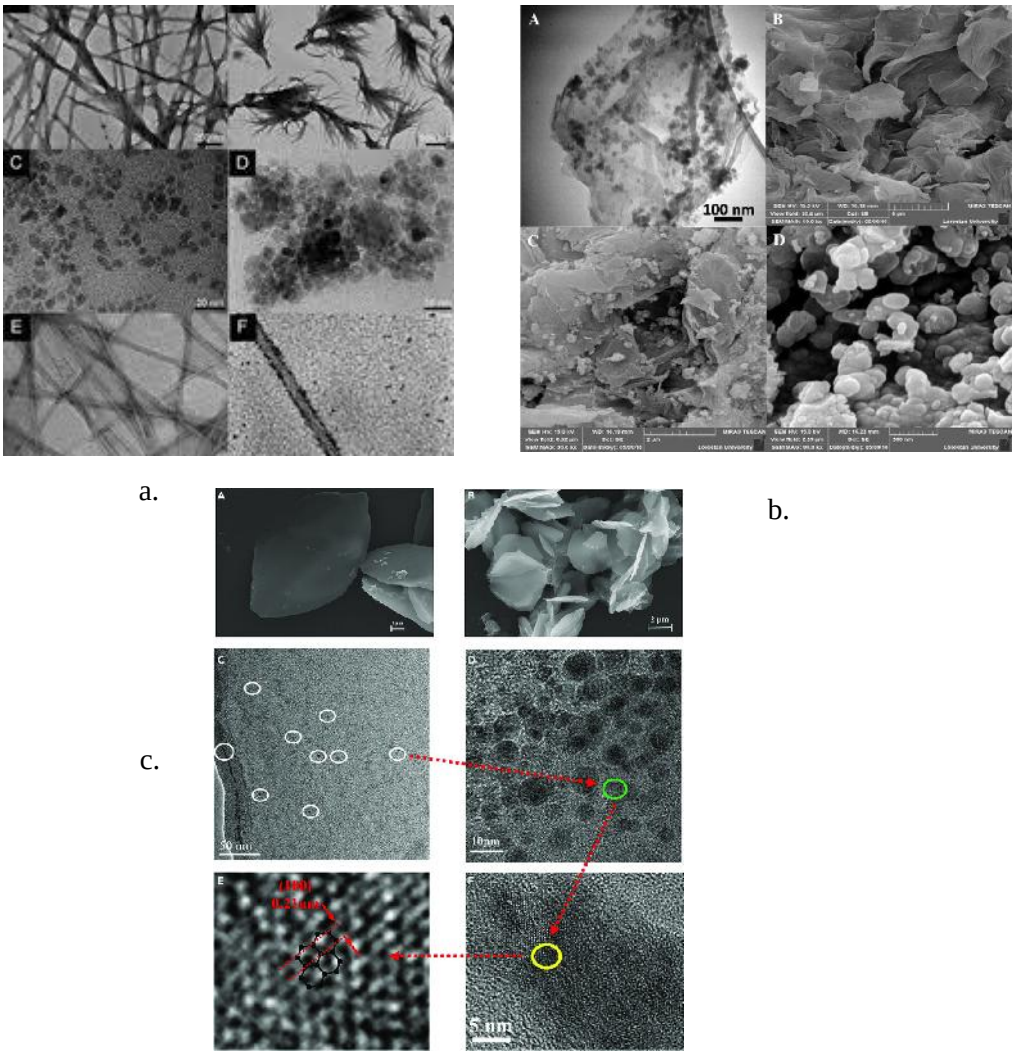



Figure-6.8 TEM Studies of of a. FPT b. LP c. FPT-LP

6.4.4 Differential Scanning Calorimetry (DSC)

DSC is a thermal analysis that measures the change in the physical properties of a sample, over variations in temperature and time. During analysis, the instrument measures the heat flow associated with thermal transitions, such as melting or crystallization, providing insights into the material's thermal properties. DSC detection involves capturing the heat flow signals to generate a thermogram, allowing for the precise characterization of phase transitions, thermal stability, and other thermal events in the sample.

The sample preparation was conducted by encapsulating the material in a hermetically sealed pan. The sample pan was carefully mounted in the DSC instrument, where it undergoes controlled heating. The obtained thermogram was compared with the thermograms of FPT, coformer and PM recorded in a similar manner under the same conditions. (Fig-6.9).

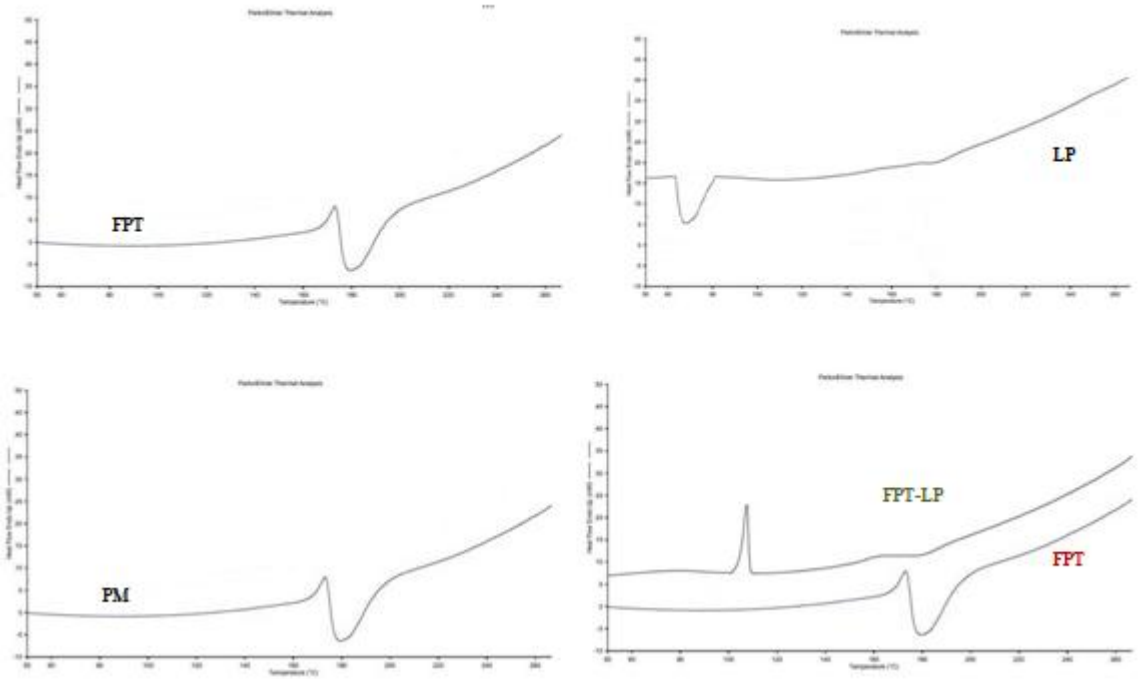



Figure-6.9 DSC Thermograms of FPT, LP, PM, Overlaid FPT and FPT-LP

6.4.5 Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

RP-HPLC is a chromatographic technique where non polar stationary phase separates analytes based on their hydrophobicity in a polar mobile phase. It is widely used for the qualitative and quantitative analysis of organic compounds. RP-HPLC method was developed and validated (Refer 5.1.4) ensuring compatibility with the stationary and mobile phase. The prepared cocrystal was dissolved in the mobile phase and injected into the HPLC system, equipped with a reversed-phase column. The sample elutes through the column based on its hydrophobicity, and detection is based on the absorption of the analyte as being eluted from the column. UV-Vis detection was employed to identify and quantify the separated compounds, providing valuable information about the chemical composition of the sample, formation of cocrystal and purity. The obtained chromatogram was compared with the chromatograms of FPT, coformer and PM recorded in a similar manner under the same conditions. (Fig-6.10).

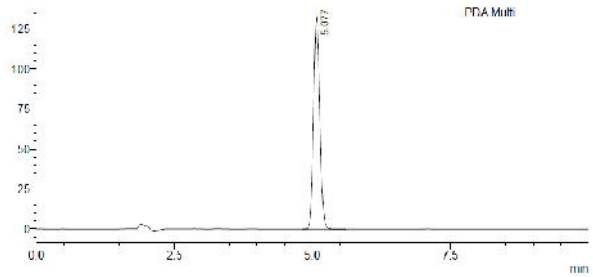



Figure-6.10 Chromatogram of FPT-LP

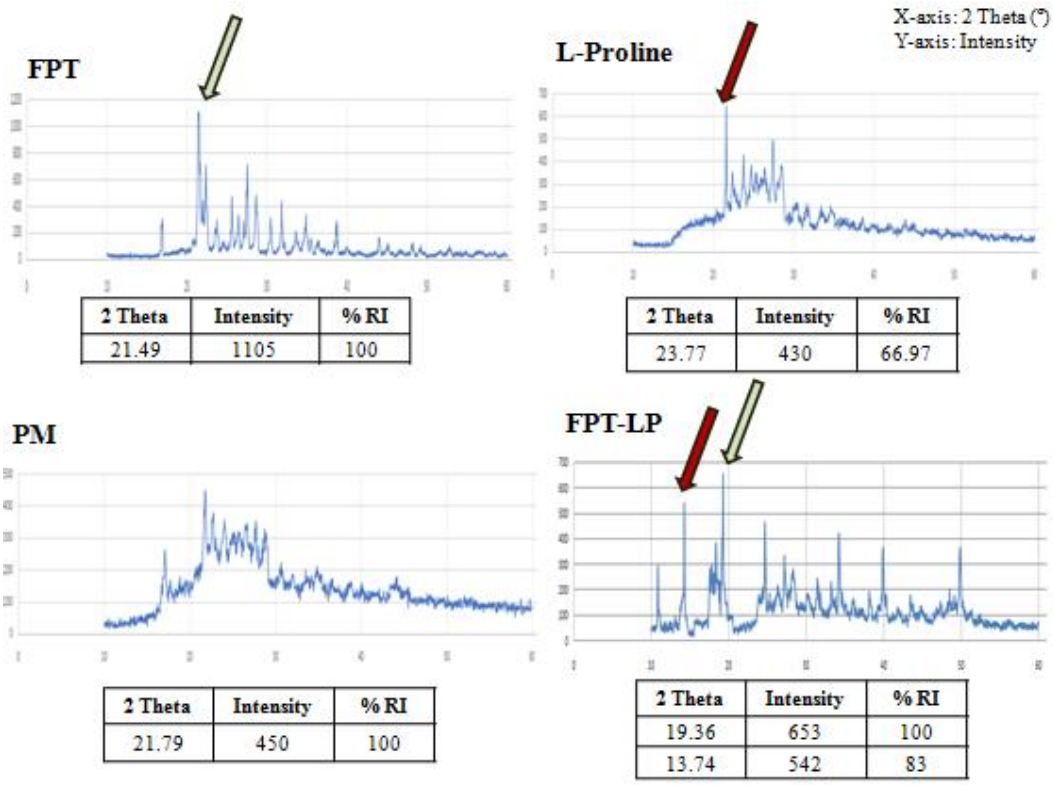
6.4.6 Powder X-Ray Diffraction (PXRD)

PXRD is a technique used to analyze the crystal structure of powdered materials by exposing them to X-rays, producing diffraction patterns. It analyzes powdered samples, useful for polycrystalline materials and phase identification. It enables quick identification of crystalline phases and quantitative analysis of the sample.

PXRD sample preparation involved grinding the sample into a fine powder to ensure a random orientation of crystallites. The powdered sample was then mounted onto a sample holder. The X-Rays were directed onto the sample with the help of the PXRD instrument software. This resulted in the diffraction pattern of the sample. The obtained diffractogram was compared with the diffractograms of FPT, coformer and PM recorded in a similar manner under the same conditions (Fig-6.11).

*Relative crystallinity ($X_c$):* Relative crystallinity is a measure of the degree of crystallinity in a material relative to its overall structure. It is often expressed as a percentage and provides information about the fraction of a material's structure that exists in a crystalline state compared to the amorphous or disordered regions. The relative crystallinity of a material can be calculated from the PXRD data using the following formula:

$Xc = I_{crystalline}/I_{total} \times 100$, where $I_{crystalline}$ is the integrated intensity/area of the crystalline peaks and $I_{total}$ is the total integrated intensity/area of all peaks in the PXRD pattern. The Xc was calculated with Origin 9 software, by taking the intensity of the PXRD into the consideration. The relative crystallinity of polymers usually ranges in between 0 % (fully amorphous) to 100 % (fully crystalline). The common crystalline substances lie in between the range of 40 % to 60 %.




| 2 Theta | Intensity | % RI |
|---|---|---|
| 21.49 | 1105 | 100 |

| 2 Theta | Intensity | % RI |
|---|---|---|
| 23.77 | 430 | 66.97 |

| 2 Theta | Intensity | % RI |
|---|---|---|
| 21.79 | 450 | 100 |

| 2 Theta | Intensity | % RI |
|---|---|---|
| 19.36 | 653 | 100 |
| 13.74 | 542 | 83 |

Figure-6.11 PXRD Diffractograms of FPT, LP, PM and FPT-LP

6.4.7 Single Crystal X-ray Diffraction (SC-XRD) Study

SC-XRD is a crystallographic method for the determination of structures at the atomic level. It analyzes a single crystal, providing precise atomic positions and reveals detailed three-dimensional structures with high precision.

The SC-XRD sample (the developed cocrystals) was preliminarily analyzed under an electron microscope to confirm the formation of single crystal (Fig-6.12). It was then mounted on a thin fiber or loop and placed in the X-ray beam path. In the SCXRD instrument, X-rays interact with the crystal lattice, generating a diffraction pattern. Detectors capture the diffracted X-rays.

Inference: The resulting data elucidated from X-rays were used to determine the three-dimensional arrangement of atoms within the crystal structure. The crystallograph with distinct peaks is showed in Fig-6.13. SC-XRD also determined the geometric configuration, symmetry, and potential intermolecular interactions within the crystal lattice as well as confirmed the formation of cocrystal.

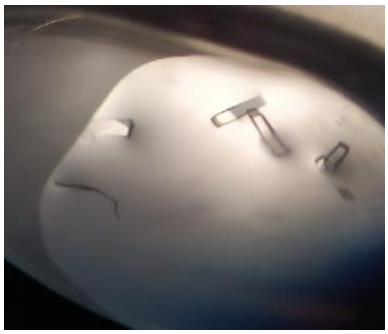


Figure-6.12 Microscopic Studies of FPT-LP for SCXRD

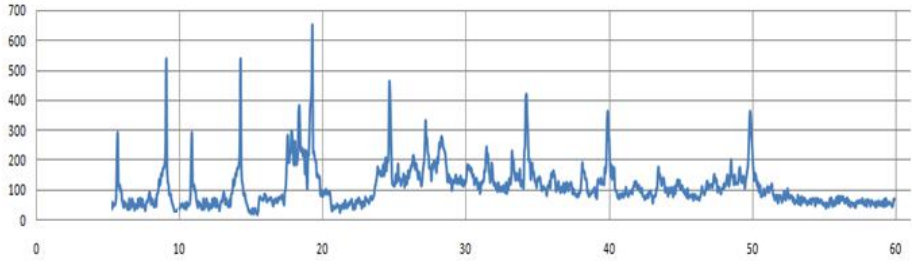



Figure-6.13 SCXRD Diffractogram of FPT-LP

**6.5 Stability of the developed cocrystals**

The stability of developed cocrystals must exhibit robust stability profiles to ensure the reliability and efficacy of the final drug product. Comprehensive stability studies are conducted to assess the chemical, thermal, and moisture stability of cocrystals. Chemical stability is critical to prevent degradation and maintain the integrity of the API and coformer over time. Thermal stability studies helps to know the cocrystals' performance under different temperature conditions, while moisture stability evaluations are essential to address sensitivity to environmental humidity. This ensures the sustained quality, safety, and efficacy of the cocrystal.

6.5.1 Phase Transformation

The Phase transformation refers to the alteration of the atomic structure and properties of a sample, often occurring with changes in temperature, pressure, or composition, leading to distinct phases or states. Phase transformation study is a type of chemical stability. The phase transformation studies of developed cocrystals FPT-LP was conducted in aqueous medium by taking 10 mg cocrystal in 10 mL distilled water. The residual solids were dried and subjected to PXRD analysis at 0 hr and after the equilibrium time of 48 hr. Fig-6.14 shows the phase transformation diffractogram by PXRD, where a is at 0 hr and b is at 48 hr.

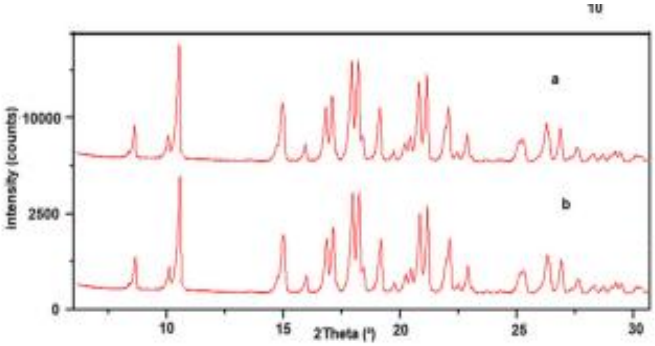



Figure-6.14 Phase Transformation Studies of FPT-LP (a) 0 hr and (b) 48 hr

6.5.2 Photostability Study

The photostability of the developed cocrystal FPT-LP was studied in a photostability chamber under U.V. light (200 Watt/$m^2$) and Visible light (1.2 million lux) with temperature (40 ± 2 °C) and humidity (75 ± 5 %) following the guidelines of ICH Q1B (R2) guidelines. The samples were placed in petridish and sealed with paraffin sheet. After the completion of Lux cycle (12 days), the sample was analysed using developed RP-HPLC method (Refer: 5.1.4) by using 15 µg/mL concentrated solution (Table-6.6).

Table-6.6 Photostability studies of FPT-LP

| Time points | Peak Area (n-3) ± SD | % RSD |
|---|---|---|
| Before starting the Lux cycle | 767726.33 ± 1328.85 | 1.80 |
| Completion of the Lux cycle | 734901.64 ± 1748.39 | 1.74 |

6.5.3 Accelerated Stability Study

The stability of the developed cocrystal FPT-LP was studied in a stability chamber under accelerated conditions with temperature (40 ± 2 °C) and humidity (75 ± 5 %) following the guidelines of ICH Q1A (R2) guidelines. FPT-LP cocrystals were subjected to accelerated conditions in a petridish covered with paraffin sheets for a period of 3 months, with analysis points at 0, 1, 2 and 3 months by the developed RP-HPLC method (Refer: 5.1.4) by taking concentration of 15 µg/mL (Table-6.7).

Table-6.7 Accelerated Stability studies of FPT-LP

| Time points (months) | Peak Area (n-3) ± SD | % RSD |
|---|---|---|
| 0 | 767726.33 ± 1328.85 | 1.80 |
| 1 | 778019.78 ± 1460.63 | 1.95 |
| 2 | 76978.49 ± 1109.78 | 1.28 |
| 3 | 787315.10 ± 1347.15 | 2.01 |
| Stability Conditions: Temperature (40 ± 2 °C) and Humidity (75 ± 5 %) | | |

## 6.6 Discussion

### 6.6.1 Computational Studies

The ability to tailor cocrystal properties for achieving specific formulation goals is essentially through coformer selection. The in-silico studies were conducted using diverse computational software to facilitate the selection of a suitable coformer with a predisposition for cocrystal formation. The techniques employed encompassed in-silico cocrystal screening, the pKa rule, cocrystal structure analysis, Hirshfield Surface Analysis (HSA), and Surface Electrostatic Potential (SEP).

The in-silico cocrystal screening revealed the formation of both intermolecular and intramolecular hydrogen bonds, highlighting differences in heat of formation and ionization potential between FPT and all the selected coformers. L-Proline, Saccharine and HCTZ showed promising intermolecular bonding. Out of all, L-Proline exhibited the most favourable intermolecular and intramolecular bonding, 1 and 1 respectively. This indicates its high potential for hydrogen bonding with FPT and hence L-Proline was considered as one of the promising coformers.

The ΔpKa analysis, differentiates between the salt and cocrystal formation. The data stated in table 6.2 showed the ΔpKa in the range of -0.51 to 2.29 throughout the coformers selected. HCTZ and L-proline possessed the lowest ΔpKa of -0.06 and -0.51 respectively. This suggested a heightened tendency for cocrystal formation with these coformers. This study gave some basic idea in selecting the coformers with the tendency of cocrystal formation. The ΔpKa rule suggests that ΔpKa less than 0 leads to cocrystal formation, while ΔpKa more than 3 forms salts; while those between 0-4 are termed as ‘salt co-crystal continuum’. Out of all the coformers HCTZ and L-Proline showed the tendency to form cocrystals.

The shortlisted coformers mainly saccharine, HCTZ and L-Proline formed cocrystals by the solvent evaporation technique during preliminary studies. The cocrystals were evaluated based on yield, quality of cocrystals and the potential for enhancing the solubility. This helped in identifying L-Proline as the promising coformer. Hence all further studies were focused on L-Proline as the coformer.

Cocrystal structure analysis of FPT-LP unveiled a monoclinic crystal system with P21/c as the space group (where P is crystal with a primitive lattice, 2 is a 2-fold rotation axis, 1 indicates a mirror plane perpendicular to the rotation axis and c is the glide plane). This provides insights into symmetry operations affecting material properties. The addition of L-Proline (LP) led to a 'sandwich-like' trimer formation with FPT in the middle and LP at the two surfaces. This is because of the unique structure of LP, facilitating binding at both ends. This accentuates the stoichiometric ratio of 1:2 (FPT: LP) obtained during experimental optimization.

HSA studies help identify bonding sites on each atom and revealed intermolecular bonding at H-H, O-H, O-O, and π-π interaction between FPT and LP, offering insights into cocrystal packing and stability. SEP studies revealed electron-rich (red cloud) and electron-poor (blue cloud) sites of interaction between FPT and LP, demonstrating the electrostatic landscape. The cocrystal formation was facilitated by the complimentary orientation of the electron rich and electron poor sites between the drug and the coformer. This study helped in concluding the hydrogen bonding sites between the FPT and LP.

Computational studies harmonized well with the results obtained from preliminary experimental trials.

### 6.6.2 Techniques for cocrystal formation

The initial phase of cocrystal preparation involved exploring various techniques with the selected coformers. All the coformers were experimentally tested keeping the computational work as the support system.

Solvent Evaporation demonstrated cocrystal formation with saccharine, hydrochlorthiazide, and L-proline coformers at varied stoichiometric ratios, solvent and its composition and heating time. Among the variations tested, FPT-L-proline exhibited the highest yield at 1:2 stoichiometric ratio by using solvent as methanol : distilled water (20:70 v/v) at 70 ºC, producing needle-shaped cocrystals as depicted in Fig-6.4 (a).

Cooling Method, despite variations in temperature and solvent, did not result in cocrystal formation across the coformer range. Similarly, Dry Grinding and Wet Grinding, with varied trituration times and stoichiometric ratios, did not induce cocrystal formation. The Centrifugation Technique, with alterations in stoichiometric ratio, centrifugation time, speed, and solvent, did not yield any cocrystals. The Freezing Technique, employing distilled water as the solvent, also did not lead to cocrystal formation.

Supercritical Fluid Extraction (SFE) was explored as a scalable alternative to Solvent Evaporation Technique. SFE's advantages, including high speed (60-120 min per sample) in comparison to traditional methods and environmentally friendly characteristics of carbon dioxide, made it a safe and greener option. FPT-LP cocrystals were produced with a yield of 61.51 % after 6 hours via Solvent Evaporation, while SFE yielded 78.23 % cocrystals within 2 hours. This highlights the efficiency of SFE in delivering better yields and high-quality, free-flowing cocrystals in an efficient manner.

### 6.6.3 Optimization of coformer and stoichiometric ratio

A comprehensive study was encompassed using various techniques which were applied to all selected coformers across different stoichiometric ratios. Notably, the FPT-LP cocrystal exhibited remarkable results, demonstrating the highest solubility with an eight fold increase (16 mg/mL) compared to FPT (2 mg/mL). In contrast, FPT-HCTZ and FPT-Saccharine cocrystals showed substantial but comparatively lower solubility enhancements, with fourfold and threefold increase, respectively. The FPT-LP cocrystal, on exploration of different stoichiometric ratios (1:1, 1:2, 2:1, 1:1.5, 1.5:1) revealed that the ratio1:2 yielded the maximum solubility and yield. This suggests that the specific combination of FPT and L-Proline in the ratio of 1:2 optimally enhances solubility and overall yield. These findings underscore the significance of selecting appropriate coformers and stoichiometric ratios in designing cocrystals with improved solubility.

### 6.6.4 Characterization of developed cocrystals

Various analytical techniques, like FTIR, SEM, TEM, DSC, PXRD and SCXRD were employed for the comprehensive characterization of the developed cocrystals.

These methods aimed to elucidate the structural, thermal, and molecular features of the cocrystals.

FTIR spectrum of the developed cocrystals was compared to standard FPT, PM, LP and FPT-LP. The spectrum showed distinct shifts in the absorption bands in the FTIR studies that demonstrated discernible alterations in the absorption band patterns, indicating the formation of a new entity (FPT-LP). Table-6.8 shows the comparative absorbance of FPT, FPT-LP, PM and LP with respect to the functional groups present in the individual molecule.

Table-6.8 Comparative FTIR Analysis

| Functional Group | Observed Wavenumbers ($cm^{-1}$) | | | |
|---|---|---|---|---|
| | FPT | LP | PM | FPT-LP |
| N-H | 3409.44 | - | 3409.44 | 3407.24 |
| O-H | - | 3430.04 | - | 3306.54 |
| C=N | 2350.00 | - | 2354.05 | 2351.36 |
| C=O | 1645.45 | 1650.23 | - | 1647.32 |
| C-S | 640.00 | - | 641.63 | 643.12 |
| C-N | - | 1250.32 | 1206.32 | 1265.26 |
| N-O | - | 1470.00 | 1408.36 | 1470.35 |

SEM studies of FPT-LP cocrystals revealed a distinctive prismatic shape, while TEM analysis confirmed the crystalline nature of the developed cocrystal without any observable defects or impurities. These microscopic studies confirm that the cocrystals are prismatic in shape and crystalline with no crystal defects.

The DSC curve exhibited an endothermic peak at 180 °C for FPT and an exothermic peak at 110 °C for the developed cocrystal FPT-LP, setting it apart from PM and LP. An exothermic effect immediately after the endothermic peaks confirms co-crystallization.

RP-HPLC further substantiated the distinct nature of cocrystal FPT-LP, displaying a sharp peak at 5.07 min, which differed from FPT (4.3 min), PM (4.28 min), and LP(7.64 min).

PXRD and SC-XRD studies displayed distinct peaks of FPT and LP in the FPT-LP diffractogram, matching the individual diffractogram peaks respectively. FPT showed 2 theta value as 21.49 with an intensity of 1105, while L-Proline showed 2 theta value as 23.77 with 430 as the intensity. PM showed 2 theta value similar to

FPT which is 21.79 with lesser intensity of 450. FPT-LP diffractogram showed the distinct peaks of both the diffractograms (FPT and LP) with the 2 theta value 19.36 and 13.74 with 653 and 542 as the intensity respectively. This concludes the formation of a new spieces (molecule) with the peculiarities of both FPT and LP, while shift in 2 theta value depicts the change in the orientation because of cocrystal formation.

The relative crystallinity is a valuable parameter as it provides insights into the structural characteristics of a material. The relative crystallinity will be a percentage indicating the fraction of the material that is in crystalline form. The relative crystallinity of the developed cocrystals was observed as 66.98 %. This states that the developed cocrystal FPT-LP (1:2) is crystalline in nature.

The combined application of FTIR, SEM, TEM, DSC, RP-HPLC, PXRD and SCXRD proved instrumental in confirming the identity and formation of cocrystals, distinguishing them from the FPT, PM, and LP.

### 6.6.5 Stability Studies of developed cocrystals

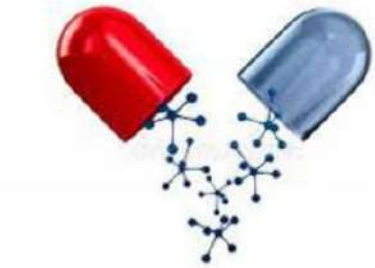
The stability studies were conducted using phase transformation studies, photostability studies and accelerated studies. This gave an overview about the stability of the developed cocrystals.

The Phase transformation study was conducted for FPT-LP in solution phase at 0 hr and 48 hr, by PXRD. This study revealed no significant changes when compared at the two time points. This provides a deeper insight into the mechanism of solution–mediated phase transformation of FPT-LP cocrystals proving the chemical stability of the developed cocrystals.

The photostability and accelerated stability studies were conducted according to ICH guidelines Q1A and Q1B respectively. This study showed no significant change in the peak area of the chromatogram, hence proving that the developed cocrystal (FPT-LP) are stable to light, temperature and moisture.

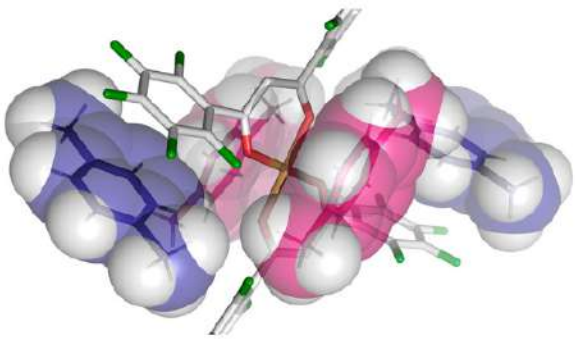
In summary, the computational studies acted as a guiding route in the selection of coformers. These coformers were then tested through experimental techniques for cocrystal formation. The optimization of stoichiometric ratio was guided by computational and experimental data, leading to the identification of an optimal ratio for FPT-LP cocrystals. The technique of cocrystal formation explored was

solvent evaporation technique, which was taken up as scale up technique by Supercritical Fluid Extraction. Characterization techniques further validated the success of cocrystal formation and provided detailed insights into their structural and molecular characteristics. Each characterization complimented each other in giving a detailed outcome of the developed cocrystal. The interlinking of computational and experimental approaches ensured a comprehensive and effective exploration of cocrystal development. Addressing the stability studies of the developed cocrystal it ensured the quality, safety, and efficacy of cocrystal-based drug formulations coming forth.



# Chapter-7

# Pharmacokinetic Study



# CHAPTER - 7

# Pharmacokinetic Study

The Pharmacokinetic (PK) study is a comprehensive examination of how the body interacts with substances administered over the entire duration of exposure to the API. Pharmacodynamics focuses on the drug's effects on the body, while PK studies focus on characterizing the Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the API, such as FPT, and its developed cocrystal, FPT-LP. Key parameters, like time to achieve maximum concentration, maximum concentration and clearance rate, are compared to gain critical insights. The pharmacokinetic studies, conducted on animals (rats) initiated with the approval of Institutional Animal Ethics Committee (IAEC) followed by development of a bio analytical Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method for both FPT and FPT-LP. This method is instrumental in precisely determining the concentrations of FPT and FPT-LP in blood plasma. The PK studies play a fundamental role in unraveling how the body processes and respond to the administered substances, offering crucial information for the bioavailability of the molecule.

## 7.1 IAEC Approval

Institutional Animal Ethics Committee (IAEC) approved the project proposal for conducting animal studies at L. M. College of Pharmacy, Ahmedabad. [Proposal Number: LMCP/IAEC/22/0024; Date of Approval: 15-February-2022; Validity: 06 months (Annexure-I)]. The Animal Study Protocol included Animal specification, Drugs or Chemicals to be administered, Blood collection route and time points, Anaesthesia and grouping of animals, etc (Tables-7.1 and 7.2). The animals selected were Sprauge-Dawley rats of either sex, 8-10 weeks old weighing 250-300 g. The study was conducted in two groups with 06 animals each. The treatment was planned with FPT and FPT-LP cocrystal for each group respectively.

Table-7.1 Animal Study Protocol for Pharmacokinetic Study

| | |
|---|---|
| Animal Specifications | Species and Strain: Sprauge-Dawley Rats of either sex<br>Age: 8-10 weeks old<br>Weight: 250-300 g<br>Total number of animals required: 12 |
| Drugs/Chemicals | FPT - API |

| | |
|---|---|
| | FPT-LP - Cocrystal |
| Anaesthesia | Isofluran |
| Blood collection route | Retro-orbital plexus |

Table-7.2 Grouping of Animals for Pharmacokinetic Studies

| Sr. No. | Group | Treatment | Administration | No of animals |
|---|---|---|---|---|
| 1 | Standard Control | FPT | Oral | 06 |
| 2 | Test Control | FPT-LP | Oral | 06 |
| Total no of animals | | | | 12 |

**7.2 Development of Bio analytical Method**

An LC-MS/MS analytical method was developed and validated for the precise estimation of blood plasma samples. The reported method for pharmacokinetic studies using rat was initially studied that was found unsuitable for use for the FPT-LP cocrystals.[74]

A new bio analytical method was developed for the analysis of FPT-LP cocrystals and FPT. Table-7.3 shows preliminary studies on optimization of bio analytical method, using varied mobile phase ratios and modes (isocratic and gradient). The bio analytical method was developed in such a way that it gives proper chromatogram and distinct peak shape of FPT, FPT-LP and Losartan (Internal Standard).

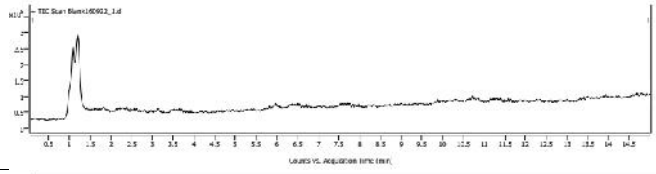
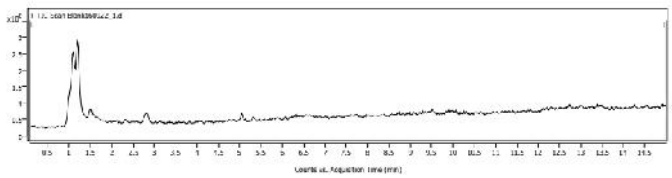
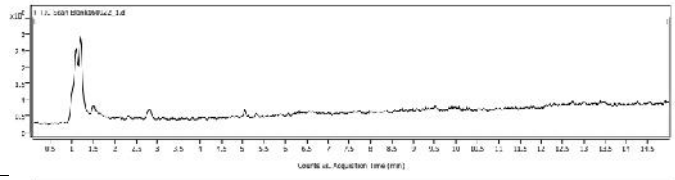
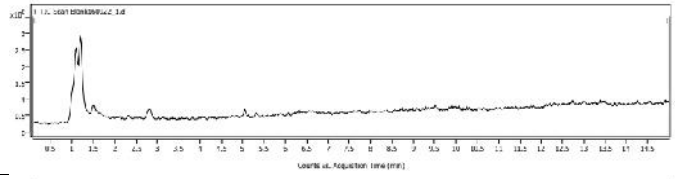
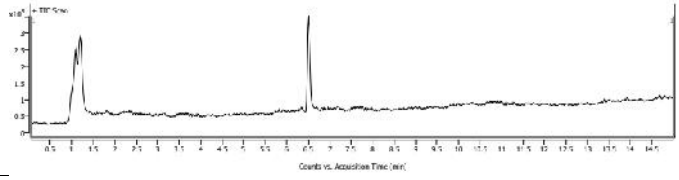
*Selection of internal standard*

The accurate and precise quantification, as well as the overall robustness of the bio analytical method, heavily relies on the careful selection of an internal standard. The internal standard selected should accurately reflect the chemistry of the specific drug under investigation, FPT. Hence, the sartan class of drug was selected. The three potential internal standards considered for investigation were Losartan, Valsartan, and Telmisartan with molecular weights of 422.91 g/mol, 435.51 g/mol, and 514.61 g/mol, respectively. The bio analytical method employed for detection was LC-MS/MS. The molecular weight of FPT was 501.62 g/mol, the internal standard selected was strategically the one with the molecular weight farthest from FPT. Consequently, Losartan was chosen as the internal standard for both FPT and FPT-LP. This careful selection aims to enhance the precision and accuracy of the quantification process while ensuring the robustness of the analytical method.

The optimized method was developed using Zorbax RP-C18 as the stationary phase (100 mm × 3.0 mm, 1.8µ) and a time gradient mobile phase A consisting of 0.1 %

formic acid in water (v/v) and mobile phase B composed of ACN (Table-7.4) at a flow rate of 0.4 mL/min. The injection volume was set at 1 µL, and the column temperature was maintained at 30 ºC for duration of the run time, namely, 10 minutes. Mass parameters were configured with an ESI source in positive mode, featuring a curtain gas at 20 psi, collision gas at 06 psi, and a temperature of 450 ºC. Fig-7.1 and 7.2 depicts mass spectra of FPT and FPT-LP respectively by LC-MS/MS. The M+ peak was obtained at 502 and 732 m/z for FPT and FPT-LP respectively Aliquots, as per the specifications in section 5.1.3, were meticulously prepared. The calibration curve, ranging from 50 to 700 ng/mL, was studied and coefficient of regression and overlay was plotted as shown in Fig-7.3 (a) and (b) respectively. The optimized conditions are stated Table-7.4 with time gradient programming of mobile phase in Table-7.5.

Table-7.3 Preliminary Optimization of LC-MS/MS Method

| Sr. No. | Mobile Phase with variable Ratios / Flow Rate | Chromatogram | Observation |
|---|---|---|---|
| 1. | Blank |  | - |
| 2.[74] | ACN:water (0.05% FA) 40:60 v/v 0.2 mL/min |  | No peak observed for FPT-LP |
| 3. | ACN:water (0.05% FA) 60:40 v/v 0.2 mL/min |  | No peak observed for FPT-LP |
| 4. | ACN:water (0.05% FA) 80:20 v/v 0.4 mL/min |  | No peak observed for FPT-LP |
| 5. | ACN:water (0.05% FA) Time gradient |  | Distinct peak was observed for FPT-LP |

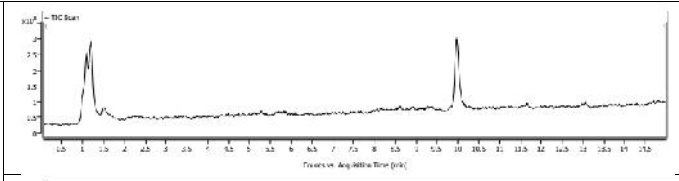
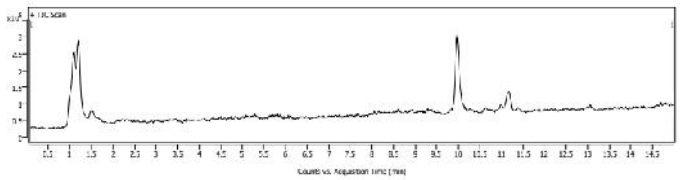
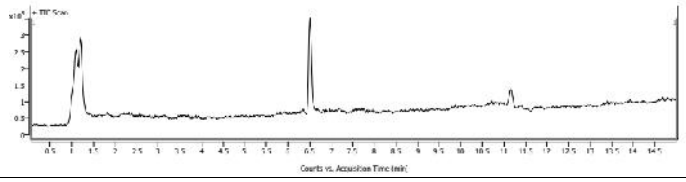
| | | | |
|---|---|---|---|
| 6. | 0.4 mL/min |  | Distinct peak was observed for FPT |
| 7. | |  | Distinct peak for FPT and Losartan |
| 8. | |  | Distinct peak for FPT-LP and Losartan |

Table-7.4 Optimized Chromatographic Conditions for LC-MS/MS

| Parameter | Conditions |
|---|---|
| Chromatographic Parameters | |
| Mobile Phase | ACN: water (0.1% formic acid) time gradient (Table-7.4) |
| Stationary Phase | Zorbax RP-C18, 100 mm × 3.0 mm, 1.8µ |
| Flow Rate | 0.4 mL/min |
| Injection volume | 1 µL |
| Column Temperature | 30 ºC |
| Run Time | 15 min |
| Mass Parameters | |
| Curtain gas | 20 psi |
| Collision gas | 06 psi |
| Temperature | 450 ºC |

Table-7.5 Time gradient programming for LC-MS/MS Method

| Time (min) | Mobile Phase A | Mobile Phase B |
|---|---|---|
| 0.00 | 60 | 40 |
| 2.00 | 50 | 50 |
| 5.00 | 20 | 80 |
| 6.00 | 20 | 80 |
| 10.00 | 60 | 40 |
| 15.00 | 60 | 40 |

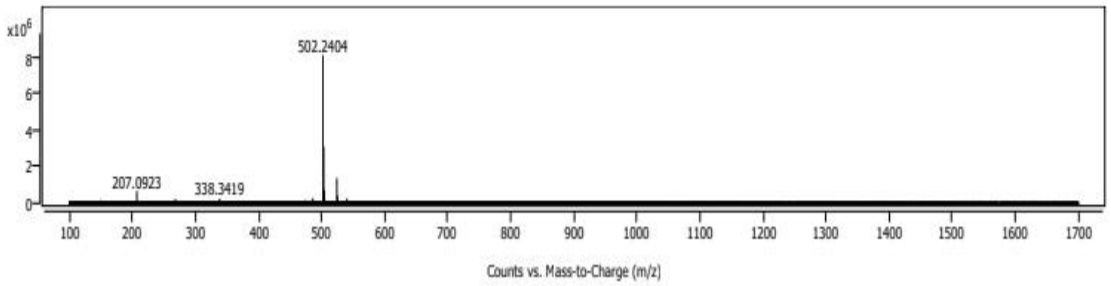



Figure-7.1 Mass spectra for FPT

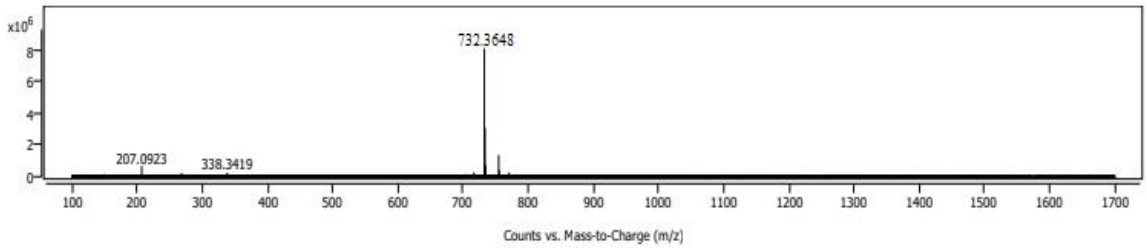



Figure-7.2 Mass spectra for FPT-LP

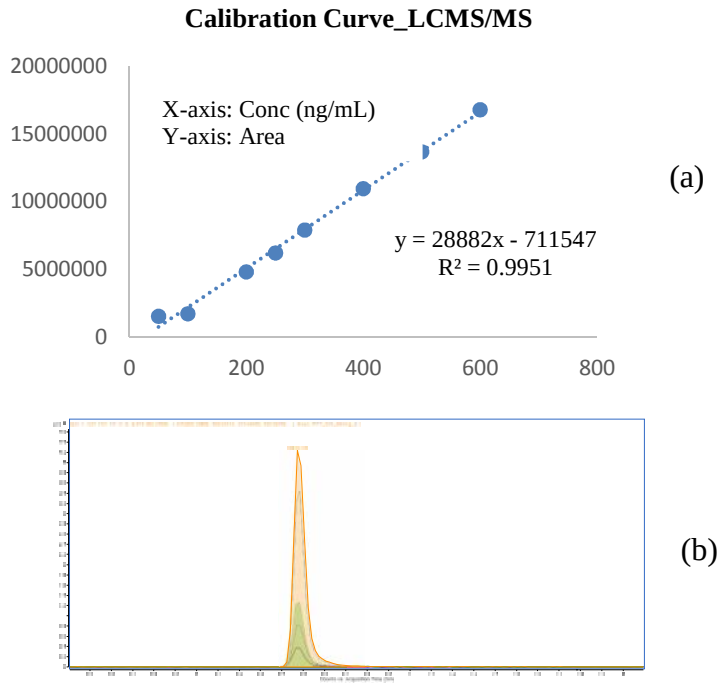



Figure-7.3 LC-MS/MS Method for FPT-LP (a) Calibration Curve (b) Overlayed Chromatogram

## 7.3 Pharmacokinetic Studies

### 7.3.1 Experimental Design

The experimental setup was assigned to two distinct groups of rat, each comprising six members: the standard control group and the test control group. The rat equivalent dose (equivalent to 60 mg) was calculated for FPT and FPT-LP, from the human dose and was in accordance with the average weight of the rats under

study. The drug was weighed and dissolved in water and administered orally.

Blood samples (less than 1 mL) were carefully withdrawn from the retro orbital plexus under anaesthesia, ensuring minimal stress. The time points for blood withdrawal were strategically chosen, covering intervals of 0, 1, 2, 2.5, 3, 3.5, 4, 8, 12, 24, and 48 hours, enabling a comprehensive assessment of the behaviour of the administered drugs over time.

The collected blood was processed, that involved treatment with 1 % EDTA solution and subsequent centrifugation at 10,000 rpm for 10 minutes, resulting in the extraction of plasma.

*Sample Preparation*

The plasma was combined with 50 µL of Losartan (at a concentration of 100 ng/mL) and was extracted with 300 µL quantity of ACN, following another round of centrifugation. The centrifugation was performed at 15,000 rpm and 4 ºC for 10 minutes.

*Optimization of extraction process*

The extraction process was optimized after various trials with addition of varied amount of ACN like 50, 100, 150, 200, 250, 300 and 350 µL. This is important for maximum extraction of the drug from the plasma. 300 µL of ACN was the optimized volume to be used for the extraction of FPT-LP (Table-7.6).

The supernatant was carefully collected. The next stage involved the analysis of the bioavailability of the drug through the developed LC-MS/MS method.

Table-7.6 Optimization of extraction process of ACN for FPT-LP

| Extraction volume (µL) | Concentration of FPT-LP |
|---|---|
| 50 | 368 |
| 100 | 1532 |
| 150 | 1324 |
| 200 | 1673 |
| 250 | 1896 |
| 300 | 2819 |
| 350 | 2817 |

7.3.2 Study of the Pharmacokinetic Parameters of FPT and FPT-LP

The sample study was undertaken at each specific time point, and a comparative study was conducted between FPT and FPT-LP, as illustrated in Table-7.6 and Fig-7.7. The evaluation of pharmacokinetic parameters was performed using the LC-

MS/MS data processed through PK Solver, yielding a comprehensive set of parameters detailed in Table-7.8. These parameters encompassed Concentration maxima ($C_{max}$), Time maxima ($T_{max}$), half-life ($t_{1/2}$), Area under the curve from 0 to t time ($AUC_{0-t}$), Volume of distribution (Vz_obs), Clearance (Cl_obs), and Steady State Volume (Vss_obs).

Table-7.7 Comparative Data of Pharmacokinetic Studies for FPT and FPT-LP

| Time (hr) | FPT-LP | | FPT | |
|---|---|---|---|---|
| | Concentration (ng/mL) (n-3) | SD ± % RSD | Concentration (ng/mL) (n-3) | SD ± % RSD |
| 0 | 0 | - | 0 | - |
| 1 | 150 | 1.23 ± 0.67 | 150 | 1.73 ± 1.16 |
| 2 | 180 | 1.15 ± 0.64 | 180 | 2.06 ± 1.12 |
| 2.5 | 260 | 1.73 ± 0.87 | 200 | 1.34 ± 0.50 |
| 3 | 320 | 2.08 ± 0.86 | 240 | 1.52 ± 0.63 |
| 3.5 | 270 | 0.57 ± 0.25 | 230 | 1.15 ± 0.50 |
| 4 | 230 | 1.03 ± 0.45 | 220 | 1.73 ± 0.79 |
| 5 | 210 | 0.57 ± 0.27 | 210 | 1.52 ± 0.72 |
| 6 | 180 | 1.73 ± 1.02 | 170 | 1.09 ±0.58 |
| 7 | 160 | 1.35 ± 0.67 | 150 | 2.36 ± 1.35 |
| 8 | 150 | 1.52 ± 1.91 | 80 | 1.52 ± 1.95 |
| 12 | 80 | 1.67 ± 1.69 | 60 | 0.57 ± 0.96 |
| 24 | 50 | 2.30 ± 1.89 | 50 | 0.53 ± 1.14 |
| 36 | 0 | - | 0 | - |
| 48 | 0 | - | 0 | - |

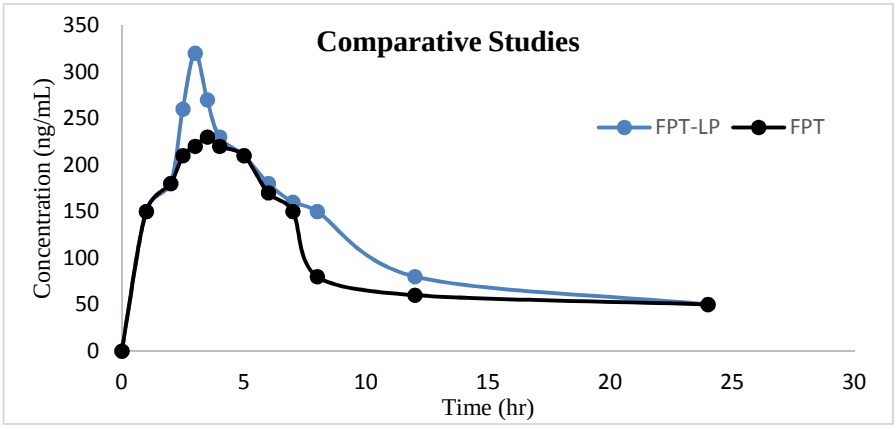



Figure-7.4 Comparative Studies of Pharmacokinetic Data

Table-7.8 Pharmacokinetic Parameters of FPT and FPT-LP

| Parameter | Unit | FPT-LP | FPT | % Change |
|---|---|---|---|---|
| $t_{1/2}$ | h | 10.68 | 8.51 | 20.29 |
| $T_{max}$ | h | 3.00 | 3.00 | 0.00 |
| $C_{max}$ | ng/mL | 320.00 | 240.00 | 25.00 |
| $AUC_{0-t}$ | ng/mL*h | 2682.50 | 2295.00 | 14.45 |
| Vz_obs | (mg)/(ng/mL) | 0.26 | 0.25 | 5.38 |
| Cl_obs | (mg)/(ng/mL)/h | 0.017 | 0.020 | -18.64 |
| Vss_obs | (mg)/(ng/mL) | 0.25 | 0.28 | -10.21 |

**7.4 Bioavailability Calculation**

The assessment of the developed cocrystals FPT-LP included the calculation of both absolute and relative bioavailability. The bioavailability was calculated using the formula $F = AUC_{0–t(test)}/AUC_{0–t(reference)} \times 100$ %. This formula compares the area under the concentration-time curve (AUC) for FPT-LP to that of FPT, providing a quantitative measure of the relative bioavailability of the developed cocrystals.

**7.5 Dose Reduction Calculation**

Dose reduction in general refers to the reduction in the amount or frequency of a medication to achieve a desired therapeutic effect while minimizing potential side effects or risks. The maximum concentration of a drug in the bloodstream ($C_{max}$) is an important pharmacokinetic parameter that represents the peak concentration reached after administration. The theoretical dose reduction is calculated from the $C_{max}$ data of FPT and FPT-LP. The following formula is used to estimate the new dose.

New Dose=Old Dose×Target Cmax/Current Cmax; where New Dose is the adjusted or reduced dose, Old Dose is the original or current dose, Target $C_{max}$ is the desired or target maximum concentration, Current $C_{max}$ is the observed or current maximum concentration.

From the pharmacokinetic studies the dose reduction for FPT-LP is 41.25 mg.

**7.6 Discussion**

The pharmacokinetic studies conducted on rats played a pivotal role in assessing the Absorption, Distribution, Metabolism, and Excretion (ADME) of the studied

molecules, FPT and FPT-LP cocrystal. Plasma samples obtained from rats were carefully processed and analyzed using the developed bio analytical method, with Losartan serving as the internal standard. FPT and FPT-LP exhibited distinct molecular weights, with m/z values of 502 and 732.36 respectively.

A reported LC-MS/MS method is available for the drug FPT.[74] The method was attempted to be used for the analysis, but peaks for FPT and FPT-LP were not detected in the chromatogram by the reported method, even after variations in the mobile phase. Hence, there was a need to develop a new bio analytical method which can analyze FPT, FPT-LP and Losartan (Table-7.9). The developed method used the same two solvents as the reported method but with time gradient programming. The chromatogram for FPT and FPT-LP showed the retention time as 10 and 6.5 min respectively, while losartan showed the retention time of 11.3 min with the developed bio analytical method (Table-7.9). The calibration curve was plotted for FPT and FPT-LP within the linearity range 50 to 700 ng/mL. This demonstrated high accuracy with a coefficient of regression of 0.9951. Partial validation of the method was done in terms of precision, accuracy and freeze thaw cycle. This advanced analytical technique provided precise insights into the concentration of the drug in the plasma samples of both the groups (FPT and FPT-LP). The plasma sample preparation was conducted with Losartan as the internal standard and extracting the drug from the plasma by meticulously trying various concentrations of ACN. 300 µL of ACN showed the maximum possible extraction of the drug from the sample. If the sample is not extracted completely it may give inappropriate quantitative results.

A comparative evaluation was conducted between FPT and FPT-LP, to shed light on potential differences in the pharmacokinetic studies. The results of the pharmacokinetic studies unveiled noteworthy differences between FPT and FPT-LP cocrystal on various parameters. FPT-LP showed a significantly higher $C_{max}$ of 320 g/L compared to FPT (240 g/L), indicating an augmented peak concentration. Moreover, the AUC for FPT-LP was substantially greater by 22 % at 2822 mg*h/L in contrast to 2295 mg*h/L for FPT, implying prolonged exposure over time. The studies further highlighted a 20.29 % increase in the half-life of FPT-LP compared to FPT, indicating a prolonged duration of the drug. The steady-state volume of distribution of FPT-LP was increased by 10.21 % and the drug concentration in plasma surged by 14.26 % in comparison to FPT. Importantly, the clearance rate

was decreased by 18.64 % for FPT-LP, suggesting increased bioavailability of the drug. These pharmacokinetic findings suggested that the developed cocrystal (FPT-LP) is more bioavailable in comparison to FPT.

The absolute bioavailability, representing the unchanged administered dose reaching systemic circulation, was determined as 25 %. The relative bioavailability, indicating the effectiveness of FPT-LP compared to FPT, was calculated at 32 %. The bioavailability data for FPT is reported as 18.6 %.[57] The comparison reveals a substantial increase in bioavailability by 88.88 % for FPT-LP, emphasizing a significant enhancement in the proportion of the drug reaching systemic circulation.

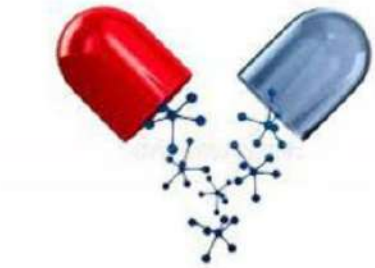
From the bioequivalence perspective the increased bioavaibility can lead to a dose reduction for the same pharmacodynamic response. The theoretical dose reduction of the developed FPT-LP cocrystal is 41.25 mg. This dose reduction of approx twenty mg (FPT dose – 60 mg) helps in minimizing the side effects associated with FPT.

Table-7.9 Comparison of the Developed and Reported Bio Analytical Methods

| Parameters | Reported Bio analytical method for FPT[74] | Developed Bio analytical method |
|---|---|---|
| Mobile Phase [Elution] | ACN : water (0.1 % v/v formic acid) [Isocratic elution] | ACN : water (0.1% v/v formic acid) [Time Gradient elution] |
| Run Time (min) | 15 | 15 |
| Retention Time (min) | 9 | 6.5 (FPT-LP), 10 (FPT), 11.2 (Losartan) |
| Resolution | - | Resolved |
| Internal Standard | BR-A-563 | Losartan |
| Application | This method is applied for FPT | This method is applied for FPT, FPT-LP with internal standard |

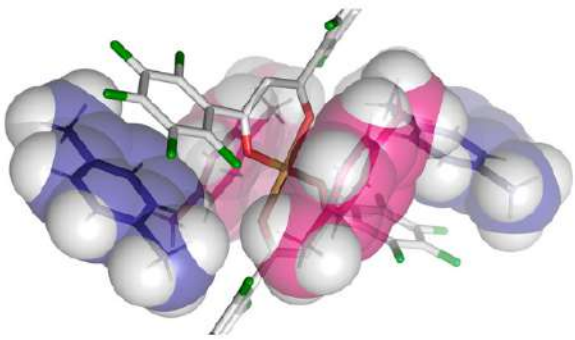
In summary, the pharmacokinetic studies showed an increase in the concentration maxima ($C_{max}$) and AUC of FPT-LP in comparison to that of FPT. The increase in other parameters like half life, steady state volume and drug concentration was also observed. There was a decrease in the clearance rate which suggests that the FPT-LP cocrystals are more bioavailable. The bioavailability study demonstrated a remarkable 88.88 % increase of FPT-LP compared to FPT, showcasing the potential of cocrystals to enhance drug absorption. Based on theoretical considerations the reduction in dose for FPT-LP is 41.25 mg; approximately two third of the present

dose of FPT. This collectively demonstrates that the developed cocrystals, FPT-LP, exhibit markedly increased bioavailability compared to FPT.



# Chapter-8

# Formulation Study



# CHAPTER - 8

# Formulation Study

Formulation study is the process of determining the best way to deliver the drug (FPT-LP). Formulation studies assess the bioavailability of a drug based on its particle size and solubility. The inactive ingredients or excipients must be added in such a way that the formulation of each dose is consistent, stable and patient compliant without any variation from one tablet or capsule to the next. Capsule formulation is suitable for cocrystals due to several advantages it offers in terms of drug delivery. It allows precise control over dosage, making it easier to tailor with cocrystal, by retaining cocrystal stability, formulation flexibility (hard and soft gelatin). The formulation studies were initiated with the identification of suitable excipients, followed by preformulation studies. The formulation studies consisted of drug-excipient compatibility studies, method of preparation, studies on varied batches/ formula, evaluation studies and stability studies with the prediction of shelf life.[41]

## 8.1 Preformulation Study

Preformulation study is an integral part of designing any formulation. It focuses on the physicochemical properties of the API.[42] This provides idea about the possible modification in the formulation to have better delivery of the API. The preformulation studies include organoleptic properties, melting point, particle size analysis, powder properties, solubility studies, in-vitro dissolution studies, and intrinsic dissolution studies.

### 8.1.1 Organoleptic Properties

The organoleptic characteristics state the colour and odour of FPT, L-Proline and FPT-LP cocrystals (Table-8.1).

Table-8.1 Organoleptic Properties of FPT, LP and FPT-LP

| Name | Colour | Odour |
|---|---|---|
| FPT | White | Odorless |
| L - Proline | White | Odorless |
| FPT-LP | White | Odorless |

### 8.1.2 Melting Point Determination

Melting point determination is crucial in assessing the purity of a substance and verifying its identity, as impurities can alter the melting point range. The melting point of FPT was determined using Veego melting point apparatus by open capillary method. The observed melting point and the reported melting point is shown in Table-8.2.

Table-8.2 Melting Point Determination of FPT, LP and FPT-LP

| Name | Reported Melting Point Range | Observed Melting Point Range |
|---|---|---|
| FPT | 270-273 °C | 271-274 °C |
| L - Proline | 250-254 °C | 252-256 °C |
| FPT-LP | - | 265-268 °C |

### 8.1.4 Particle Size Determination

The particle size determination not only focuses on the physical properties of solid drugs but also on their biopharmaceutical behaviour. It works on the basic principle of smaller the particle size, larger the surface area for absorption.

The particle size was determined by wet analysis. The sample was prepared by admixing 10 mg FPT-LP in 10 mL of TWEEN 80. The medium of analysis was water. Parameters were set at Absorption index: 0, RPM: 2000, Sonication Time: 120 min, Obscuration Range: 0-20 %. After the saturation of the system, the prepared sample was added in the medium and results were recorded. The results were collected in n=3. The particle size and it's distribution is shown in Fig-8.5. It determines the size as volume mean diameter of 90% of the total volume in the sample (d(v)90), d(v)50 and d(v)10.

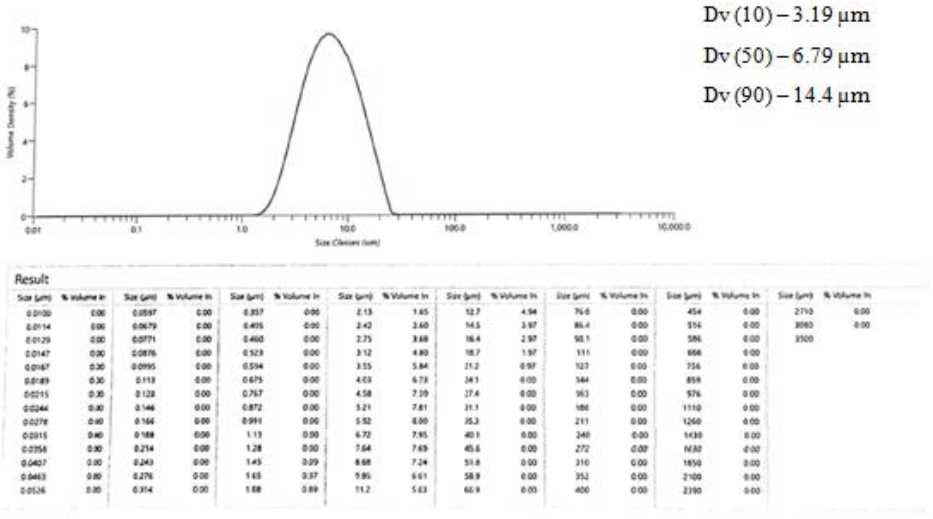



Figure-8.1 Particle Size Analysis of FPT-LP

8.1.5 Powder Properties

The flow properties of powders are of utmost importance when preparing dosage forms efficiently. Powder properties play a vital role in various aspects including processability, flowability, packing and storage, uniformity, homogeneity, dissolution and solubility, mechanical strength, and stability. By understanding and controlling these powder properties, manufacturers can optimize their processes, enhance product quality, and ensure consistent and reliable performance.

8.1.5.1 Bulk Density

Bulk density, a critical property for characterizing powdered materials, quantifies the mass of a powder in relation to its total volume. Bulk density has a significant influence on the flowability of the powder. .

The sample was passed through a sieve with apertures greater than or equal to 1.0 mm. The weighed 250 mg sample was gently introduced into a dry graduated cylinder of 50 mL without compacting. The unsettled volume or the bulk volume (BV) was noted and the bulk density was calculated.

Formula: D = M /BV, where: D: Bulk density (g/l) M: Weight of the full container (g) BV: Container volume in bulk (l).

8.1.5.2 Tapped Density

Tapped density is a property used to measure the packing efficiency and compaction behavior of powdered materials. It refers to the density of a powder when it is subjected to tapping or vibration to reduce the void spaces between particles. Tapped density is an important parameter during compaction.

The sample was passed through a sieve with apertures greater than or equal to 1.0 mm. The weighed 250 mg sample was gently introduced into a dry graduated cylinder of 50 mL without compacting. The graduated cylinder was tapped 100 times and the tapped volume (TV) was noted and the tapped density was calculated with formula using the TV.

Formula: D = M / TV, where: D: Tapped density (g/l) M: Weight of the full container (g) TV: Tapped Container volume (l)

8.1.5.3 Angle of Repose

The angle of repose, of a granular material is the angle of dip that is relative to the horizontal plane on which the material is piled and the angle of the material on the slope face is on the verge of sliding. The angle of repose can range from 0° to 90°.

The angle of repose was studied by fixing the funnel in such a way that it is close to the flowing cone. The sample was poured through a funnel to form a cone. The pouring of the material is stopped when the pile reaches a predetermined height. The angle of the resulting cone was measured directly, by dividing the height by half the width of the base of the cone. The inverse tangent of this ratio is the angle of repose.

Formula: Angle of repose = tan-1(2h/d), where h is the height of the pile of the powder and d is the Diameter of the cone.

8.1.5.4 Carr's Index and Hausner's Ratio

The Carr's Index and the Hausner's Ratio are the indicators of the flowability of bulk solids by studying its compressibility.

Formula: Carr_Index = $(\rho_{tapped}-\rho_{bulk})/\rho_{tapped}*100$;

Hausner's Ratio = $\rho_{tapped}/\rho_{bulk}$; $\rho_{tapped}$ : the tapped bulk density of the material ($kg/m^3$), $\rho_{bulk}$ : the loose bulk density of the material ($kg/m^3$)

8.1.6 Solubility Studies

Solubility studies play a vital role in the pre-formulation studies as the therapeutic efficacy of the drug depends on its solubility. In order for a drug to enter the systemic circulation and exert a therapeutic effect, it must first be in solution. Relatively insoluble compounds often exhibit incomplete absorption.

10 mg of FPT, PM and FPT-LP were added in 3 different volumetric flasks containing 5 mL distilled water and shaken for 10 min on shaker. Distilled water was added upto the mark of the volumetric flask (10 mL) and shaken for further 10 min. The resulting solution was filtered using Whatman filter paper (1; 110 mm). The filtrate was then analyzed. The filtrate was analyzed using the reported U.V. visible

spectroscopy method (Refer: 5.1.3). Table-8.3 shows the data about concentration in 10 mL and solubility in water.

Table-8.3 Solubility Studies of FPT, PM and FPT-LP

| Sample | Absorbance (n-3) | Concentration in 10 mL | Solubility in water (mg/mL) |
|---|---|---|---|
| FPT | 0.3741 ± 0.14 | 10.06 | 6.66 ± 0.53 |
| PM | 0.4036 ± 0.17 | 12.43 | 7.18 ± 0.72 |
| FPT-LP | 0.6501 ± 0.08 | 18.14 | 11.56 ± 0.28 |

8.1.7 Dissolution Method

Dissolution method assesses the rate at which the API dissolves in a specific dissolution medium under controlled conditions. The dissolution apparatus selected is IP-II (Basket type). FPT, PM and FPT-LP equivalent to single dose (60 mg) was placed in the basket, rotated at 100 rpm where the dissolution medium was Phosphate buffer pH-6.8, 900 mL and the bath temperature was 37 ± 0.5 ºC for 60 min. The aliquots (5 mL) of the medium are withdrawn at specific intervals (0, 10, 20, 30, 45, 60 min) and analyzed to measure the amount of substance dissolved by the developed RP-HPLC method.

8.1.8 Intrinsic Dissolution Rate

The intrinsic dissolution rate (IDR) is defined as the rate of dissolution of a pure pharmaceutical active ingredient by keeping other parameters constant like stirring speed and ionic strength of the dissolution medium. This acts as an important parameter to study the release pattern of the API and to predict the formula.

The study method is as described in section 8.1.7. The sample interval for IDR study was 0, 20, 40, 60, 80, 100, 120, 140 and 160 min (Table-8.4, Fig-8.2).

Table-8.4 Intrinsic Dissolution Rate Study of FPT, LP, PM and FPT-LP

| Time (min) | 0 | 30 | 60 | 90 | 120 | 150 | **IDR mg/cm²/min** |
|---|---|---|---|---|---|---|---|
| FPT (%) | 0 | 29.62 | 59.82 | 69.42 | 81.71 | 84.37 | 16.87 |
| PM (%) | 0 | 29.90 | 62.02 | 70.01 | 83.48 | 86.15 | 17.23 |
| FPT-LP (%) | 0 | 47.93 | 85.63 | 93.74 | 99.76 | 102.43 | 20.48 |

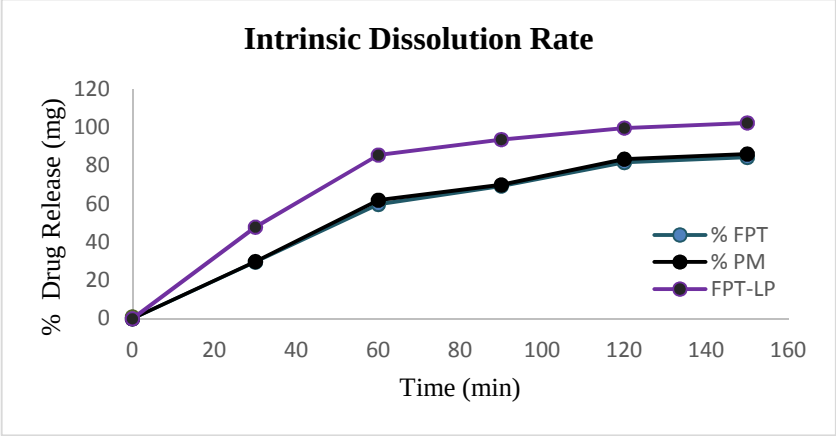



Figure-8.2 Intrinsic Dissolution Rate of FPT, PM and FPT-LP

8.1.8 In-vitro Dissolution Study

In-vitro dissolution study is the % Cumulative Drug Release with respect to time. The objective is to evaluate the in vivo performance of the drug that affects the rate and extent of release of a drug substance from the finished dosage form.

The In-vitro dissolution study was performed as described in section 8.1.7.

The percentage cumulative drug release for the FPT, PM and the FPT-LP is as shown in Table-8.5 and Fig-8.3.

Table-8.5 In-vitro Dissolution Studies of FPT, PM and FPT-LP

| Time (min) | % Cumulative Drug Release | | |
|---|---|---|---|
| | FPT | PM | FPT-LP |
| 0 | 0 | 0 | 0 |
| 10 | 23.20 ± 0.51 | 25.12 ± 2.47 | 32.21 ± 1.27 |
| 20 | 55.56 ± 1.47 | 58.13 ± 0.53 | 71.73 ± 2.21 |
| 30 | 69.43 ± 1.58 | 72.17 ± 1.31 | 89.21 ± 3.41 |
| 45 | 82.01 ± 2.24 | 83.15 ± 3.42 | 95.07 ± 1.24 |
| 60 | 85.20 ± 2.28 | 85.57 ± 1.39 | 99.31 ± 2.81 |

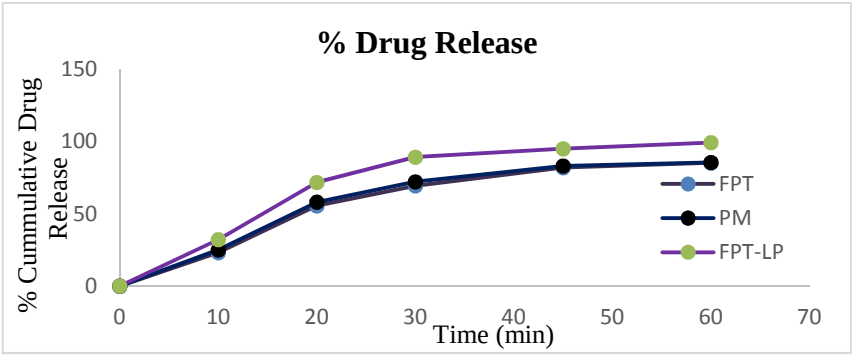



Figure-8.3 In-vitro Dissolution Study of FPT, PM and FPT-LP

## 8.2 Preparation and Evaluation of Dosage Form

### 8.2.1 List of Excipients

Excipients are additives that have no therapeutic activity but are added to the formulation along with pharmacologically active substance. The main purpose of adding the excipients is to increase the bulk of the formulation along with imparting desired properties for better patient compliance.

The list of excipients used for the preparation of capsule dosage form of FPT-LP are showed in Table-8.6

Table-8.6 List of Excipients Used for the Formulation Study

| Name of the Excipient | Category | Strength (% w/w) | Source |
|---|---|---|---|
| Microcrystalline Cellulose (MCC) (Avicel pH 101) | Diluent | 20-90 | Cadila Healthcare |
| Sodium Starch Glycolate (SSG) | Disintegrant | 2-8 | Otto Chemicals |
| Cross Carmallose Sodium (CCS) | Disintegrant | 10-15 | Otto Chemicals |
| Aerosil | Glidant | 0.5-5.0 | Loba Chemie |
| Magnesium Stearate (Mg. Stearate) | Lubricant | 0.25-5.0 | High Purity Laboratory Chemicals |

### 8.2.2 Drug Excipient Compatibility Study

#### 8.2.2.1 Hygroscopicity Study

Hygroscopicity is the extent of absorption of moisture from the atmosphere by the compound. The absorption of moisture can increase the rate of degradation, reduce potency, generate toxic metabolites and deform the formulation during storage. The drug excipient hygroscopicity was studied at 40 ± 2 °C and 75 % RH for 30 days in a stability chamber. The results of the study are depicted in Table 8.7.

Table-8.7 Hygroscopicity Study of Drug and Drug Excipient Mixture

| Sr. No. | Sample Constituents | Initial Weight (mg) | Final Weight (mg) | Difference (mg) | % Change (w/w) |
|---|---|---|---|---|---|
| 1. | FPT-LP Cocrystal (Closed) | 21.134 | 21.142 | 0.008 | 0.038 |
| 2. | FPT-LP Cocrystal (Open) | 22.237 | 22.258 | 0.021 | 0.095 |
| 3. | FPT-LP + MCC | 22.300 | 22.308 | 0.008 | 0.046 |

| | | | | | |
|---|---|---|---|---|---|
| 4. | FPT-LP + SSG | 21.416 | 21.425 | 0.009 | 0.042 |
| 5. | FPT-LP + CCS | 22.317 | 22.325 | 0.008 | 0.035 |
| 6. | FPT-LP + Aerosil | 21.225 | 21.232 | 0.007 | 0.033 |
| 7. | FPT-LP + Mg. Stearate | 21.293 | 21.300 | 0.007 | 0.034 |
| 8. | FPT-LP + Mixture of all Excipients | 22.237 | 22.258 | 0.021 | 0.095 |

8.2.2.2 Drug Interaction Study by FTIR

The drug interaction study was determined by FTIR, as every molecule has a fixed fingerprint region and it changes with change in its composition. So, the FTIR of FPT-LP cocrystal mixed with the mixture of all excipients was performed at Day 0 and Day 30 after keeping the sample in accelerated condition in a stability chamber at 40 ºC and 75 % RH. Fig-8.4 and Fig-8.5 show the FTIR Spectrum recorded on Day 0 and Day 30 respectively.

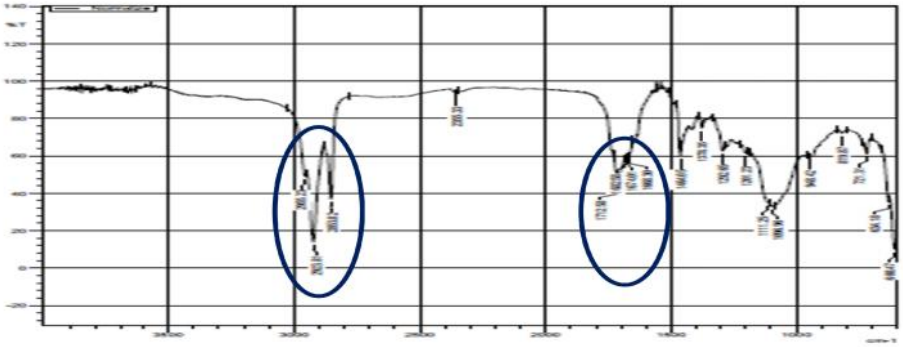


Figure-8.4 FTIR spectrum of FPT-LP and all excipients at Day 0

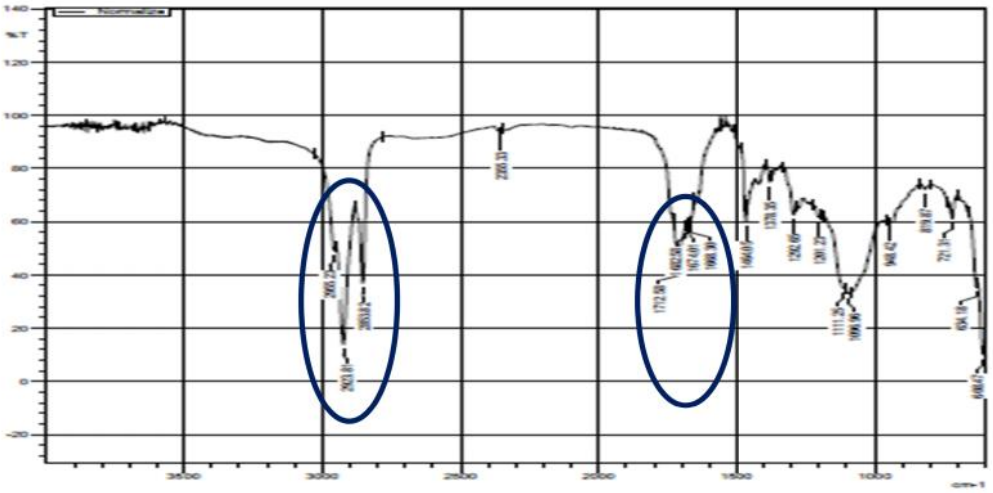


Figure-8.5 FTIR spectrum of FPT-LP and all excipients at Day 30

8.2.2.3 Potency Study

Potency study was undertaken to check the compatibility between the FPT-LP and excipients individually and all together. In other words the samples were assayed by the developed RP-HPLC method. The results of the study are shown in Table 8.8

Table-8.8 Potency Study of FPT and all Excipients

| Sr. No. | Sample Constituents | % Assay |
|---|---|---|
| 1. | FPT-LP Cocrystal | 99.1 |
| 2. | FPT-LP + MCC | 99.4 |
| 3. | FPT-LP + SSG | 99.5 |
| 4. | FPT-LP + CCS | 99.6 |
| 5. | FPT-LP + Aerosil | 99.9 |
| 6. | FPT-LP + Mg. Stearate | 98.7 |
| 7. | FPT-LP + Mixture of all Excipients | 98.2 |

8.2.2.3 DSC Study

The interaction study of FPT-LP and all excipients were conducted by DSC for the compatibility study between the cocrystal and the excipients (Fig-8.6).

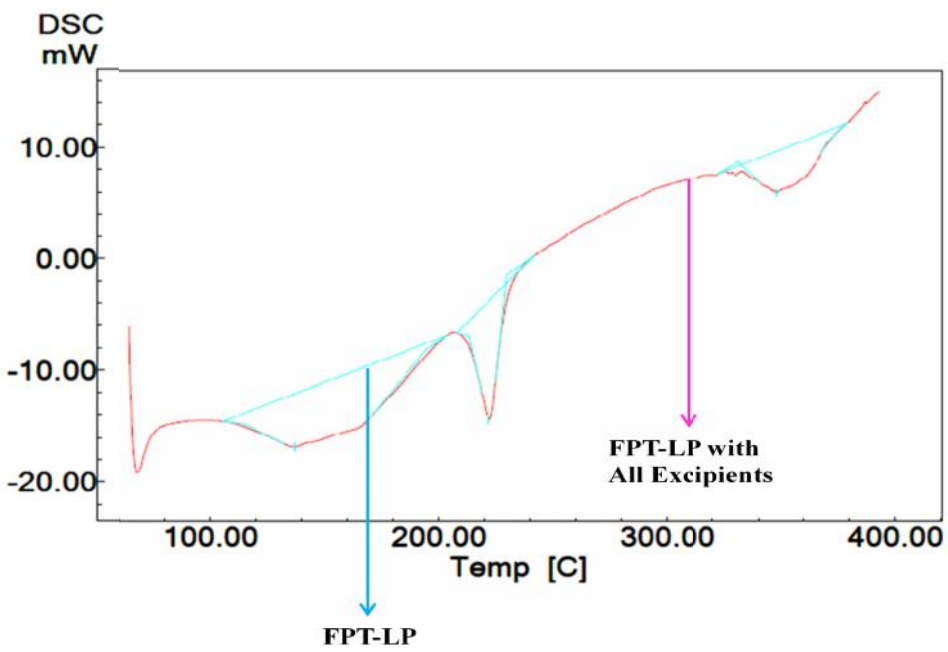



Figure-8.6 Overlay DSC of FPT-LP and FPT-LP with all excipients

8.2.3 Preparation of Dosage form

The preparation of dosage form plays a vital role in enhancing the solubility and bioavaibility of the drug. There are various excipients which play an important role

in finalising the drug formula. There is a patent for tablet formulation available, which was used as a base for the capsule formula to be optimized.[75] The selection of appropriate disintegrant with the right strength helps in promoting the drug dissolution and enhances bioavaibility.

8.2.3.1 Preliminary Batches

8.2.3.1.1 Selection of Disintegrant

The importance of disintegrant is in their ability to promote drug dissolution and enhance bioavailability by facilitating the release of API from the dosage form. Disintegrant achieve this by promoting the disruption of the capsule matrix, leading to faster disintegration and subsequent dissolution of the drug. The choice of an appropriate disintegrant is critical in the formulation process, as it directly impacts the performance and therapeutic efficacy of the oral dosage forms.

The preliminary batches were formulated for the selection of appropriate disintegrants like Sodium Starch Glycolate (SSG) and Cross Carmalose Sodium (CCS) in varying quantities over a range of 2 to 8 % and 2 to 5 % respectively. Table-8.9 depicts the formula of the different batches from F1 to F5.

Table-8.9 Selection of Disintegrant for Capsule Dosage Form

| Ingredients | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| | FPT | SSG-2 % | SSG-8 % | CCS-2 % | CCS-5 % |
| FPT | 60.00 | - | - | - | - |
| FPT-LP | - | 87.50 | 87.50 | 87.50 | 87.50 |
| MCC | 109.00 | 81.50 | 69.50 | 81.50 | 75.50 |
| SSG | 4.00 | 4.00 | 16.00 | - | - |
| CCS | - | - | - | 4.00 | 10.00 |
| Aerosil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Mg. Stearate | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Total | 180 | 180 | 180 | 180 | 180 |
| All quantities are in mg. FPT-LP was taken in a quantity equivalent to 60 mg of FPT | | | | | |

8.2.3.1.2 Pre-filling Studies

The powder mixtures prepared according to the formula indicated in Table 8.9 were

subjected to pre-filling studies to evaluate Carr's index, Hausner's ratio and the angle of repose. These studies guided the selection of the formula to be taken further for the preparation of the capsule formulation. The results of these studies are given in Table 8.10

8.2.3.1.3 Capsule Dosage Form

The capsules were prepared using manual capsule filling machine. The capsules used were hard gelatine capsules of size-1. FPT-LP and all excipients were individually weighted precisely before passing them through Sieve 80 for uniform particle size. The resulting powders were blended together, excluding the lubricant and glidant initially. The lubricant and glidant were introduced in the final stage of blending to ensure a homogenous mixture. The carefully measured and blended powder (180 mg), consisting of FPT-LP and all excipients, was then filled into each capsule body. Subsequently, the capsules were sealed with caps, finalizing the encapsulation process and producing a standardized capsule formulation.

8.2.5 Capsule Evaluation

8.2.5.1 Dissolution Method for Capsule Dosage form

Dissolution method assesses the rate at which the API dissolves in a specific dissolution medium under controlled conditions from the dosage form. The dissolution apparatus selected is IP-II (Basket type). Capsule containing cocrystal FPT-LP equivalent to single dose (60 mg) was placed in the basket, rotated at 100 rpm where the dissolution medium was Phosphate buffer pH-6.8, 900 mL and the bath temperature was 37 ± 0.5 ºC for 60 min. The aliquots (5 mL) of the medium are withdrawn at specific intervals (0, 10, 20, 30, 45, 60 min) and analyzed to measure the amount of substance dissolved by the developed RP-HPLC method. This method is used further for all the dissolution studies of the dosage form.

The formation of FPT-LP capsules were then evaluated with different parameters like weight variation, content uniformity, assay, friability, disintegration time and dissolution time (Table-8.11). Out of all the batches, F3 batch showed promising results. (Table-8.12 and Fig-8.7).

Table-8.10 Pre-filling Studies of the Powder Mixture of all Batches

| Parameters | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Carr's Index | 12.35 | 17.57 | 17.92 | 17.00 | 18.88 |
| Hausner's Ratio | 1.14 | 1.24 | 1.21 | 1.20 | 1.23 |

| | | | | | |
|---|---|---|---|---|---|
| Angle of Repose | 26.70 | 27.01 | 27.62 | 27.31 | 27.72 |

Table-8.11 Evaluation of Capsule Formation of all Batches

| Parameter | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Weight variation | 180.15 ±0.58 | 180.22 ±0.64 | 180.05 ±0.77 | 180.25 ±0.24 | 180.85 ±0.94 |
| Assay | 98.5 | 99.75 | 99.31 | 99.56 | 98.90 |
| Content uniformity | 99.4 ± 1.32 | 99.4 ± 0.87 | 99.1 ± 0.41 | 99.2 ± 1.13 | 98.9 ±0.64 |
| Friability | 0.24 | 0.22 | 0.19 | 0.21 | 0.24 |
| Disintegration Time (min) | 5±2 | 4±1 | 1±2 | 3±2 | 8±1 |
| Dissolution (30 min) | 76.89 | 84.33 | 89.68 | 85.95 | 79.68 |
| Dissolution (60 min) | 89.58 | 95.61 | 99.46 | 94.02 | 96.58 |

Table-8.12 % Drug Release for different batches of FPT-LP

| Time (min) | % Cumulative Drug Release | | | | |
|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 23.98 | 29.05 | 37.12 | 28.71 | 27.1 |
| 20 | 64.11 | 61.94 | 75.42 | 60.3 | 57.05 |
| 30 | 83.89 | 84.33 | 91.68 | 85.95 | 79.7 |
| 45 | 92.8 | 90.23 | 98.32 | 93.1 | 93.3 |
| 60 | 96.58 | 95.61 | 99.46 | 94.02 | 96.6 |

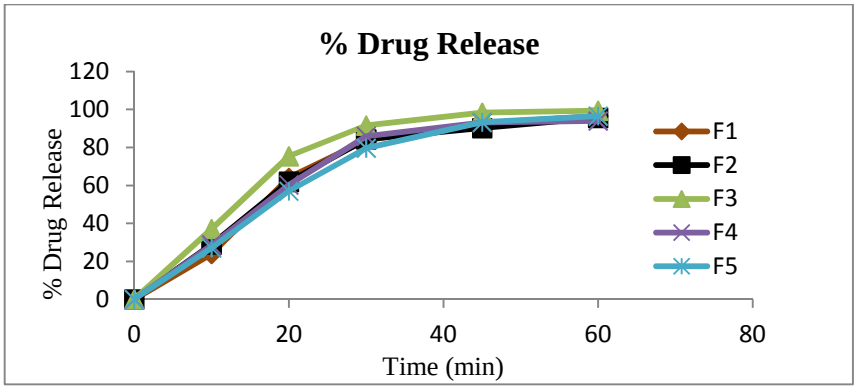



Figure-8.7 Comparative % Drug Release for different batches of FPT-LP

8.2.6 Selection of the Dissolution Medium

The saturated solubility studies of the pure FPT and FPT-LP were performed in aqueous medium-water, 0.1 N HCl and phosphate buffers pH-7.4 and 6.8. Fig-8.8 depicts the dissolution data (mg/mL) and acted as a finalising factor for the determination of optimised dissolution medium for further in-vitro studies.

Table-8.13 Comparative % Cumulative Drug Release of FPT, PM, FPT-LP and Marketed Formulation

| Time (min) | % Cumulative Drug Release | | | |
|---|---|---|---|---|
| | FPT | PM (mg) | FPT-LP (Capsule) (mg) | Marketed Formulation (mg) |
| 0 | 0 | 0 | 0 | 0 |
| 10 | 23.2 | 21.1 | 40.6 | 32.5 |
| 20 | 49.56 | 43.13 | 72.05 | 51.3 |
| 30 | 67.43 | 65.17 | 91.3 | 68.7 |
| 45 | 79.01 | 78.15 | 99.76 | 81.32 |
| 60 | 85.21 | 83.57 | 99.95 | 88.26 |
| All quantities are in mg. FPT-LP was taken in a quantity equivalent to 60 mg of FPT. All the readings were taken as n-6. | | | | |

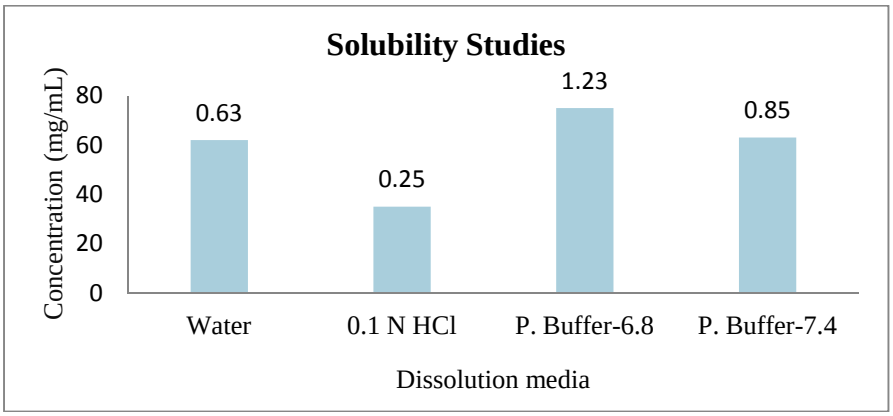



Figure-8.8 Solubility Studies for selection of Dissolution media

8.2.7 Comparative Dissolution Studies

Comparative Dissolution Studies was conducted between FPT, PM, FPT-LP Cocrystal Capsule and marketed tablet formulation (Table-8.15 and Fig-8.9).

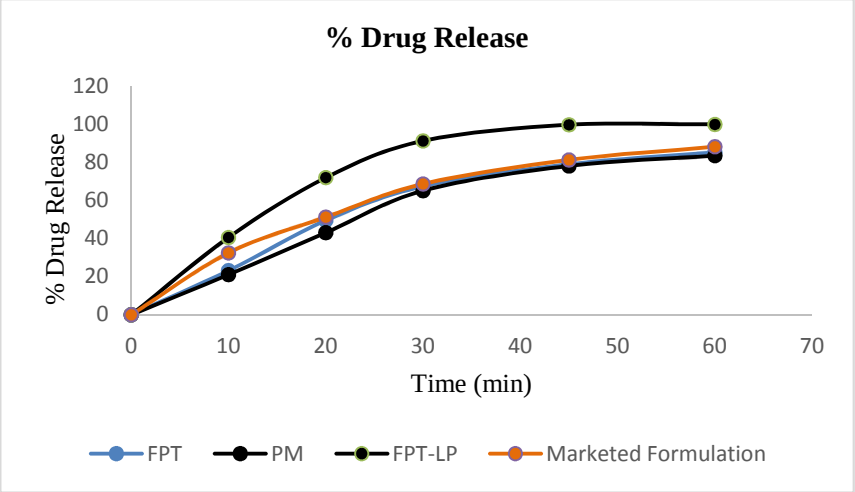



Figure-8.9 Comparative Dissolution Studies

8.2.8 Final Formula

The final formula for FPT-LP cocrystal in capsule dosage form was arrived at after considering the type and amount of disintegrant used and the results of the various preformulation studies (Table-8.14).

Table-8.14 FPT-LP Capsule Formula

| Ingredients | Amount (mg) |
|---|---|
| FPT-LP (equivalent to 60 mg of FPT) | 87.50 |
| Microcrystalline Cellulose | 69.50 |
| Sodium Starch Glycolate | 16.00 |
| Aerosil | 2.00 |
| Mg. Stearate | 5.00 |
| Total | 180 |

## 8.3 Stability Studies

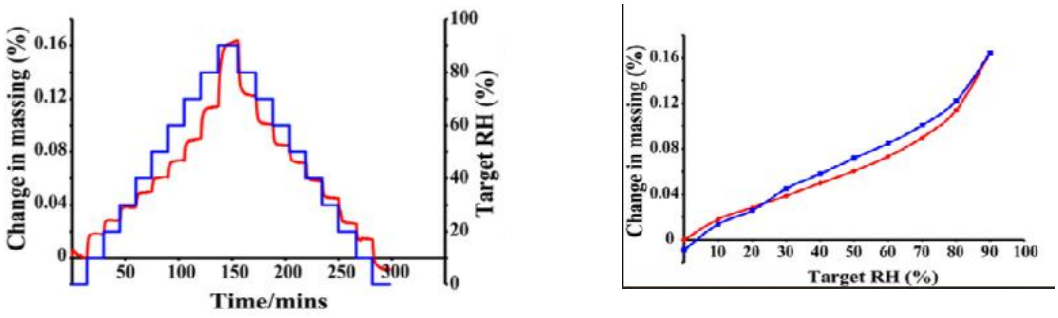
Stability studies are a critical aspect of pharmaceutical development and are conducted to assess the integrity, quality and performance of a drug formulation over time under various environmental conditions. These studies are essential to ensure that the pharmaceutical product maintains its efficacy, safety, and quality throughout its shelf life.

8.3.1 Dynamic Vapour Sorption (DVS) Analysis

Dynamic Vapour Sorption (DVS) analysis is a technique used to study the interaction between a material (API) and water vapor. This method is particularly useful for characterizing the water sorption and desorption properties of the API.

This provides insights into the moisture-related properties. This information is crucial for quality control, product development and optimizing storage conditions.

DVS was carried out using a Surface Measurement System DVS Resolution (SMS, England) at 25 °C. It measures the amount and how fast the water can be absorbed and desorbed from a sample. 100 mg of FPT-LP was dissolved in 50 mL water and placed into the sample compartment. The nitrogen flow rate was 200 mL/min. The sample was equilibrated at each of the targeted % RH (40, 80, 120 %) for an equilibration time of 3 h. Fig-8.10 (a) shows DVS change in mass plot while Fig-8.10 (b) shows Isotherm plot.




(a) (b)

Figure-8.10 DVS Graph (a) Change in the Mass Plot (b) Isotherm Plot

8.3.2 Accelerated Stability Studies

The stability of the formulation containing FPT-LP was studied in a stability chamber under accelerated conditions with temperature (40 ± 2 °C) and humidity (75 ± 5 %) following the guidelines of ICH Q1A (R2) guidelines. This study was carried out to investigate the stability of the formulation and predict the shelf life. Capsules containing FPT-LP were subjected to accelerated conditions for a period of 3 months, with analysis points at 0, 1, 2 and 3 months by the developed RP-HPLC method (Refer: 5.1.4). Table-8.15 depicts the various parameters studied during the study period.

*Shelf life Prediction:* Shelf life determination from accelerated stability studies involves extrapolating the degradation kinetics observed at elevated temperatures to estimate the time it takes for a product to reach a specified level of degradation at normal storage conditions (ICH Q1E). The Arrhenius equation is commonly used for this purpose: $\ln(k)=\ln(A)-RTEa$ (where k is the rate constant, A is the pre-exponential factor, Ea is the activation energy, R is the gas constant, and T is the absolute temperature). By plotting the natural logarithm of the rate constant against the reciprocal of temperature,

the slope (-Ea/R) allows determination of the activation energy.

Table-8.15 Accelerated Stability Studies of Capsule containing FPT-LP

| Sr. No. | Properties | Timeline (months) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| 1 | Weight variation (mg) | 192.05 ± 0.77 | 191.78 ± 0.52 | 191.23 ± 0.86 | 191.75 ± 0.74 |
| 2 | Assay (%) | 99.31 | 99.01 | 99.17 | 99.28 |
| 3 | Content uniformity (%) | 99.1 ± 0.4 | 99.7 ± 0.8 | 99.5 ± 0.3 | 99.6 ± 0.1 |
| 4 | Friability (g) | 0.19 | 0.21 | 0.19 | 0.26 |
| 5 | Disintegration Time (min) | 1 ± 2 | 1 ± 2 | 1 ± 2 | 1 ± 2 |

## 8.4 Discussion

8.4.1 Preformulation and Formulation Studies

Preformulation studies, followed by formulation studies, play a vital role in identifying and finalizing the exact formula of a dosage form along with its properties. Various preformulation studies were conducted for FPT-LP to obtain a comprehensive understanding of its physicochemical properties, facilitating the determination of an appropriate formulation and delivery method. These studies included organoleptic assessments, melting point determination, particle size analysis, powder property evaluations, solubility studies, intrinsic dissolution rate measurements, and in-vitro studies.

Organoleptic studies revealed that the colour of FPT, FPT-LP, and LP was white, and the samples were odorless.

The melting point determination states that the observed melting point was within the range of reported melting point for FPT (271-274 °C) and LP (252-256 °C). The melting point range of FPT-LP (265-268 °C) was observed to be in between the melting range of FPT and LP. This indicated the formation of a new spieces other then FPT and LP.

Particle size analysis played a crucial role in determining the size for optimal availability, with FPT-LP exhibiting specific diameter values. The particle size of FPT-LP cocrystal showed volume mean diameter of 90 % of the total volume in the

sample d(v)90 as 14.4 µm, 50 % of the sample d(v)50 showed 6.79 µm and 10 % of the sample d(v)10 in the sample posses 3.19 µm. Hence, the particle size of FPT-LP is 14.4 µm. This states that smaller particles tend to have higher dissolution rates due to the larger surface area to volume ratio and therefore, a better chance for faster absorption which gives higher bioavaibility.[32]

Powder property assessments, bulk density, tap density, angle of repose, Carr's index, and Hausner's ratio, portrayed fine powder characteristics for FPT-LP.

The solubility studies were conducted taking distilled water as the solvent. The solubility of FPT-LP was the highest with 11.56 mg/mL in comparison to PM and FPT with 7.18 and 6.6 mg/mL respectively. The IDR studies for FPT-LP were the highest (20.48 %) in comparison to PM (17.23 %) and FPT (16.87 %).

The percentage cumulative drug release of cocrystal was the highest of 99.31 % at 60 min in comparison to FPT and Physical Mixture which was 85.20 % and 85.57 % respectively. The percentage drug release graph showed Spring and Parachute effect which is typically seen during the drug release of cocrystal.

*Spring and Parachute Effect*:

Cocrystals often exhibit unique mechanical and thermodynamic characteristics, such as improved stability and solubility, which can be symbolically compared to the ability of a spring to form and deform its original shape and is known as "spring effect". This flexibility is crucial in pharmaceutical formulations, where the cocrystals' stability and the ability to maintain their structure during processing and storage are important for drug effectiveness. On the other hand, the "parachute effect" represents the ability of cocrystals to slow down or modulate the release of a drug. Cocrystals can influence the dissolution and release rates of API, similar to a parachute. This controlled release is valuable in drug delivery systems, allowing for a more predictable and sustained therapeutic effect.

The importance of these effects in cocrystal development lies in their potential impact on drug efficacy and bioavailability. Understanding and harnessing these "spring and parachute effects" in cocrystal formulations can contribute to the development of more effective and reliable pharmaceutical products.

The aqueous biological medium becomes supersaturated with the hydrophobic drug molecules that leads to higher energy (as compared to crystalline drug) which is known as the "Spring" which immediately precipitates to give loosely aggregated clusters. In order to get the benefits from this supersaturated state, it has to be

maintained for a sufficient period of time for absorption. It may require a inhibition of precipitation by using pharmaceutical excipients that intervene with growth of crystal; this refers to the “Parachute” or “precipitation inhibitors”(can either be excipient or coformer). This phase lasts for variable time to give high dose solubility.
Changes in Sample Condition: Amorphous clusters – Metastable polymorph of drug – Stable form

It can be concluded that the cocrystals of FPT-LP showed the promise of enhancing the bioavaibility of FPT when formulated into a suitable formulation.

8.4.2 Preparation and Evaluation of Dosage Form

Compatibility studies were conducted to assess the behavior of FPT-LP and excipients together in the formulation. These studies included hygroscopicity assessments, FTIR studies, DSC studies, and potency studies.

Hygroscopicity studies demonstrated negligible moisture absorption, indicating minimal hygroscopicity and non absorbent nature of FPT-LP in the presence of all the excipients.

FTIR studies over 30 days revealed consistent peaks, affirming stability of FPT-LP.

Potency studies showed no significant change by the assay study, confirming the stability of FPT-LP in the presence of all the excipients.

The DSC study indicated no interaction between cocrystal and excipient at higher temperature as the peak of FPT-LP was clearly distinguished between 320-380 ºC.

The selected dosage form was capsules, prepared using a manual capsule filling machine. Capsule formulation offers flexibility in accommodating the individual components (API and coformer) and cocrystal to retain their own crystallinity. It also has several advantages to offer in terms of drug delivery and it more considerable over tablet dosage form. Excipient selection was crucial, and various batches were prepared by varying the type and strength of the disintegrant.

Pre-filling studies affirmed excellent powder flowability for all the batches. Capsules prepared for all the batches were evaluated for weight variation, content uniformity, assay, friability, disintegration time, and dissolution time. F3 batch (Sodium Starch Glycolate 8 %) emerged as the optimal batch that showed maximum percentage cumulative drug release of 99.46 % at 60 min and fast disintegration time of 1 min.

The selection of the dissolution medium was conducted by performing saturated studies with aqueous medium- distilled water, 0.1 N HCl and phosphate buffers pH-7.4 and 6.8. The data depicts that 0.1 N HCl showed lowest solubility (0.251 mg/mL) in comparison to water (0.63 mg/mL), phosphate buffer pH 7.4 (0.85 mg/mL) where as phosphate buffer pH 6.8 showed the maximum solubility of 1.234 mg/mL for FPT-LP. The same observation was made for FPT and PM. Hence, Phosphate buffer pH-6.8 was selected as the dissolution medium for further in-vitro analysis. Table-8.16 shows the optimized dissolution parameters used throughout the finished product dissolution study.

Table-8.16 Optimized Dissolution Parameters

| **Parameters** | **Condition** |
|---|---|
| Apparatus Type | IP-II/ Basket Type |
| RPM | 100 |
| Temperature | 37 °C |
| Dissolution medium | Phosphate buffer pH-6.8 |
| Dissolution Volume | 900 mL |
| Time | 60 min |

Comparative dissolution studies were conducted for capsule formulation of FPT, PM, FPT-LP and marketed tablet formulation. The formula used for making the capsule of FPT and PM was the same as that used for the capsule of FPT-LP. FPT-LP capsule displayed the highest % drug release at 60 minutes of 99.95 %, while FPT, PM and marketed tablet formulation showed lesser percentage drug release of 85.21 %, 83.57 % and 88.26 % respectively.

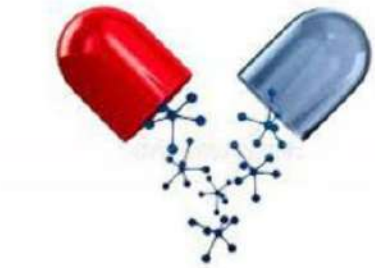
### 8.4.3 Stability Studies

Stability studies were conducted using Dynamic Vapor Sorption (DVS) analysis and accelerated stability studies. The DVS analysis presented low moisture uptake of 0.16 % at 90% RH. The low moisture uptake may only correspond to surface absorption. The desorption curve was close to the absorption curve for FPT-LP, thereby indicating that the two processes - absorption and desorption are reversible.

The accelerated stability study was conducted for 3 months in a stability chamber (40 $^{o}$C, 75 % RH) which showed negligible change in the performance characteristics of the formulation during the study tenure. The stability samples after analysis on different parameters indicated that the FPT-LP capsule is stable at high temperature and humidity. The predicted shelf life of the FPT-LP capsule is 2 years which is the

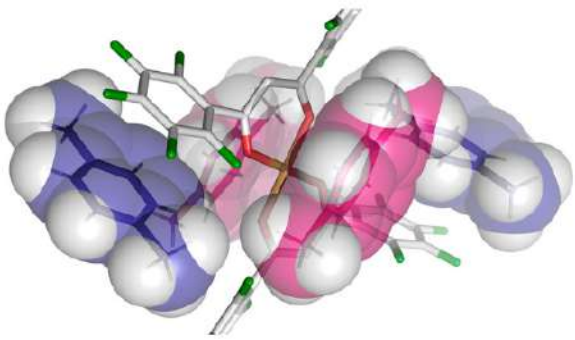
same as the marketed tablet formulation of FPT.

The preformulation and formulation studies, coupled with stability assessments, underscore the robustness and potential therapeutic efficacy of the developed FPT-LP cocrystal as a promising substitute for FPT in a capsule dosage form with equivalent stability.



# Chapter-9

# IVIVC Study



# CHAPTER - 9

# IVIVC Study

The In-vitro In-vivo Correlation (IVIVC) study defined by the U.S Food and Drug Administration (FDA) is a predictive mathematical model describing the relationship between the in-vitro property of an oral dosage form and relevant in-vivo response. It works on the principle to estimate the magnitude of the error in predicting the in-vivo bioavailability results from in-vitro dissolution data. There are four correlation levels termed as Level A, B, C, and D. Level A is highest level point to point correlation. Level B uses statistical moments, Level C uses one-point dissolution and correlates it with mean PK parameter and Level D is a rank order and qualitative analysis. The first step is conducting in-vivo studies and deconvoluting the data into time versus percentage drug release. Later the in-vitro studies is conducted and both the data sets are correlated and percentage similarity is calculated. A variety of softwares are available to co-relate the in-vitro and in-vivo data.

## 9.1 In-vivo Studies

In-vivo studies, involve experiments conducted on animals to observe biological processes or test the effects of the API. The in-vivo experiment helps in understanding the pharmacokinetics (drug absorption, distribution, metabolism and efficacy) of the API. Ultimately, in-vivo studies serve as a critical bridge between preclinical investigations and the development of safe and effective treatments for human use.

The in-vivo studies showed the estimation of bioavailability from the animal models (rats) by pharmacokinetic studies. The plasma collected from the rats was extracted for the drug and then the concentration of the drug was determined by LC-MS/MS. The In-vivo data was de-convulated to get Absorption Rate vs. Time (min) correlation (Table-9.1 and Fig-9.1).

Table-9.1 In-vivo Studies of FPT-LP

| Time (min) | Concentration (mg/mL) | Absorption Rate |
|---|---|---|
| 0 | 0 | 0 |
| 60 | 150 | 5.01 |
| 120 | 180 | 5.19 |

| | | |
|---|---|---|
| 150 | 260 | 5.52 |
| 180 | 320 | 5.63 |

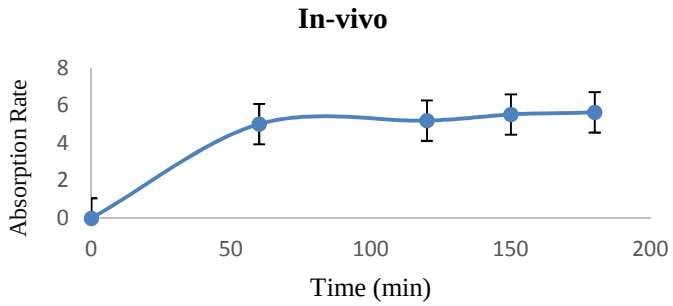



Figure-9.1 In-vivo Studies of FPT-LP

**9.2 In-vitro Studies**

The in-vitro studies play a fundamental role in assessing the characteristics and performance of pharmaceutical formulations before advancing to more complex and costly in-vivo studies. These studies, conducted in controlled laboratory settings, provide crucial insights into the behavior of the formulations at the molecular level. It helps in utilizing the in-vitro models to evaluate factors such as drug release profiles, dissolution rates and stability under various conditions. Additionally, in-vitro studies aid in the identification and alleviation of potential issues related to solubility, bioavailability and compatibility.

The In-Vitro drug release pattern was determined by dissolution studies with percentage drug release profile from 0-180 min. The graph of % drug release vs. time was plotted. (Table-9.2 and Fig-9.2)

Table-9.2 In-vitro Studies of FPT-LP

| Time (min) | % Drug Release |
|---|---|
| 0 | 0 |
| 10 | 28.20 |
| 15 | 40.60 |
| 20 | 61.82 |
| 30 | 72.05 |
| 45 | 82.13 |
| 60 | 91.30 |
| 90 | 99.76 |
| 120 | 99.82 |
| 180 | 99.96 |

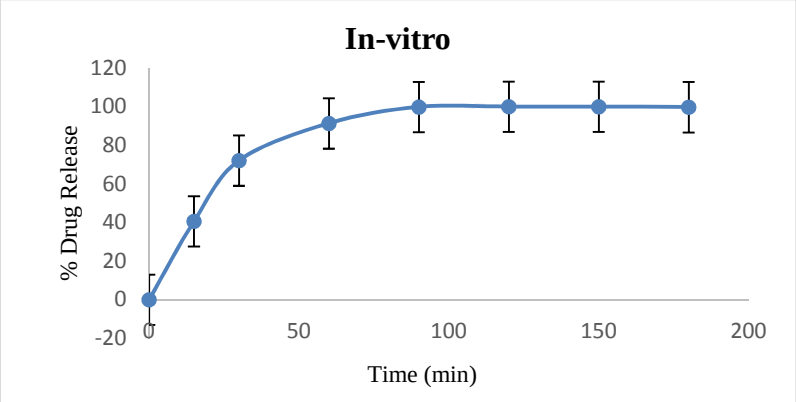



Figure-9.2 In-vitro Studies of FPT-LP

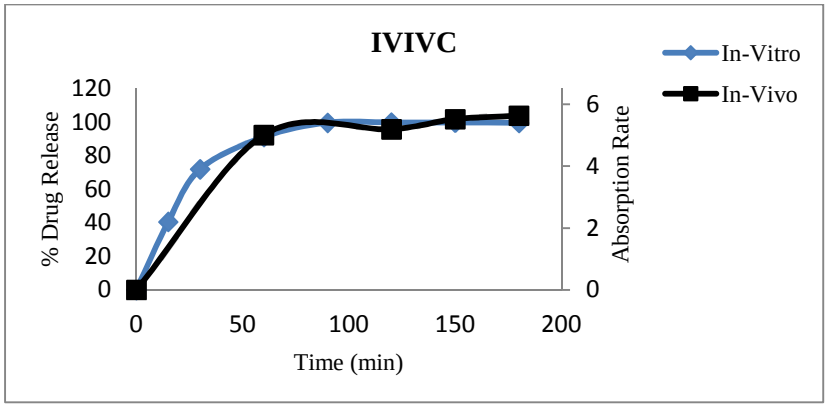
## 9.3 IVIVC Studies

IVIVC studies involve establishing a meaningful relationship between in-vitro dissolution profiles of a drug formulation and its in-vivo performance in experimental animals. These studies are crucial as they provide a scientific basis for predicting the in-vivo behavior of a drug from in-vitro data. By correlating the rate and extent of drug release in dissolution tests with the pharmacokinetic profile of the drug in the experimental animals, IVIVC helps streamline the drug development process. Successful IVIVC can facilitate more efficient quality control, enable robust bioequivalence studies, and enhance the understanding of the impact of the formulation on its therapeutic effects.

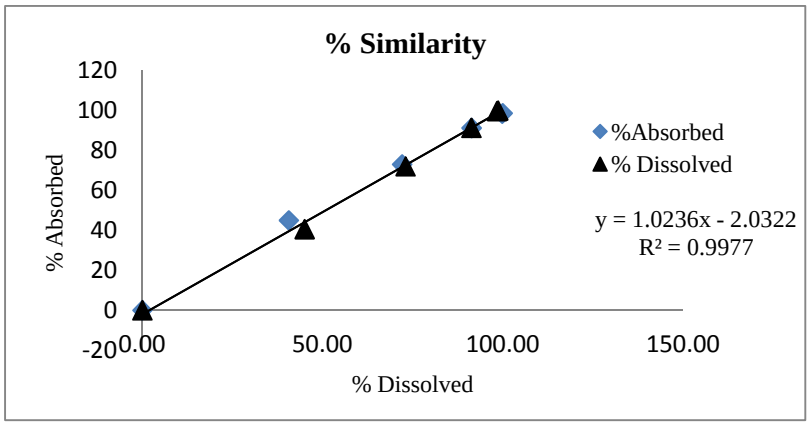
The IVIVC study was conducted by taking the data sets of both in-vitro and in-vivo studies. These data sets were compared on a single X-axis (time) with different Y-axis (% Drug Release and % Absorption Rate). The coefficient of regression was calculated using MS Excel tool (Table-9.3 and Fig-9.3).

Table-9.3 IVIVC Data

| In-vitro | | In-vivo |
|---|---|---|
| % Drug Release | Time (min) | Absorption Rate |
| 0 | 0 | 0 |
| 91.12 | 60 | 5.01 |
| 99.85 | 120 | 5.19 |
| 99.95 | 150 | 5.52 |
| 100.02 | 180 | 5.63 |




(a)




(b)

Figure-9.3 Graph of IVIVC data
(a) IVIVC Correlation (b) % Similarity

**9.4 Discussion**

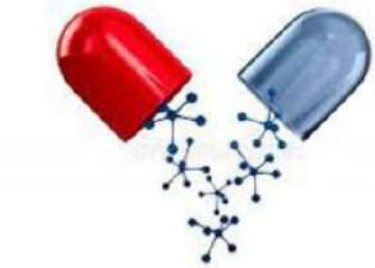
IVIVC can assign in-vivo meaning to the in-vitro data. This is a useful tool for rational development and evaluation process to reduce development time and optimize the formulation for clinical studies.[8] IVIVC correlation for cocrystals is especially important for the drugs belonging to BCS Class-II because these drugs are highly permeable and their absorption usually is limited by the dissolution.[7]

The IVIVC study was conducted by taking the time vs. concentration in-vivo data and deconvoluting it into time vs. absorption rate. This step is vital because deconvulation enables the generation of comparable data between in-vitro and in-vivo studies.

Correlational analysis, involves statistical techniques to assess and quantify the relationship between in vitro and in vivo data. A correlation analysis was used to explore the relationship between dissolution and absorption rates of cocrystals. A linear correlation ($R^2$ -0.9977) was observed between dissolution and absorption

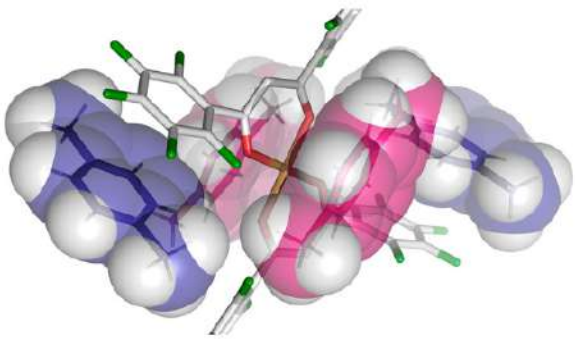
rates. When the in-vitro and in-vivo data sets were compared it showed a point to point co-relation between percentage drug Release and Absorption Rate. This indicates the highest level of correlation corresponding to Level-A correlation. This level of correlation means that the individual data points from in-vitro studies directly correspond to individual data points from in-vivo studies. Level A IVIVC is particularly advantageous in the formulation development process. It aids in selecting the most promising formulations early in development, optimizing drug delivery systems and ensuring that the final product exhibits the desired pharmacokinetic profile.

The results of the correlation analysis indicated that the FPT-LP cocrystals with a higher dissolution rate would result in a better bioavailability.



# Chapter-10

# Summary and Conclusion



# CHAPTER - 10

# Summary and Conclusion

## 10.1 Summary

About 60-70 % drugs used presently belong to BCS class II (High permeability and Low solubility). Poorly soluble drugs have limited absorption and low bioavailability resulting in higher dosage. Hence solubility enhancement is the major challenge.

Cocrystal formation with a suitable coformer offers the potential of improved solubility via modification of the underlying crystal structure, thus potentially rendering the compound bioavailable.[1] According to USFDA, "solids that are crystalline materials composed of two or more molecules in the same crystal lattice are termed as cocrystal."[2] The major advantage of cocrystal formation is the changes in physicochemical properties of the drug without altering the pharmacological properties

Fimasartan Potassium Trihydrate (FPT) is an anti-hypertensive molecule belonging to BCS class II that is low solubility and high permeability. It possesses a low bioavailability of 18.6 %. This leads to higher doses with the potential for greater side effects. Hence, it was thought of interest to design, synthesize and evaluate pharmaceutical cocrystal of FPT.

The coformer selected for making the FPT cocrystal was L-Proline, an amino acid. Amino acids are natural compounds with low risk and chiral properties, facilitating easy hydrogen bonding with FPT. L-Proline is recognized as Generally Regarded as Safe (GRAS). It is considered as an excellent candidate in cocrystal formation because it forms α-ammonium carboxylate, which support atomic interactions. It also forms head-to-tail charge-assisted hydrogen-bonded chains during cocrystal formation, thereby increasing the percentage of strong interactions.

FPT-LP cocrystals showed promising results when prepared by solvent evaporation technique and Supercritical Fluid Extraction (SFE) as the scale up technique with good yield, in stoichiometric ratio of 1:2 for FPT and L-Proline respectively.

Computational studies were employed to gain theoretical insights into the likelihood of cocrystal formation, sites of interaction and hydrogen bonding, shedding light on the underlying molecular interactions. The techniques employed were in silico cocrystal screening, the pKa rule, cocrystal structure analysis, Hirshfield Surface

Analysis (HSA), and Surface Electrostatic Potential (SEP). These studies showed the intermolecular hydrogen bonding (amino-nitro) and π-π stacking for the formation of cocrystal. It proved the presence of sandwich-like trimer upon addition of L-Proline, due to the unique structure of LP, facilitating binding at both ends. This accentuates the stoichiometric ratio of 1:2 (FPT: LP) obtained during experimental optimization.

A variety of techniques were attempted for the formation of FPT cocrystals such as Solvent Evaporation Technique, Cooling Method, Dry Grinding Method, Wet Grinding Method, Centrifugation Technique, Freezing Technique and Supercritical Fluid Extraction. All the selected coformers (like hydrochlorthiazide, saccharine and a varied aminoacids) were studied using these techniques through variations like solvent, combination of solvent, temperature, solvent volume, freezing time, cooling time, centrifugation speed, centrifugation time and stoichiometric ratios with FPT.

The developed cocrystals were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Ultraviolet (UV) spectroscopy, High-Performance Liquid Chromatography (HPLC), Powder X-ray Diffraction (PXRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Single Crystal X-ray Diffraction (SCXRD). These analysis provided detailed information about the crystalline structure, physical properties and molecular arrangement within the cocrystals; thus confirming the formation of cocrystals of FPT-LP (1:2) beyond doubt.

The results of solubility study demonstrated that the formed cocrystals exhibited a significant eight fold increase in the solubility of FPT. This enhanced solubility was a critical advantage, as it directly influences the drug's bioavailability and therapeutic efficacy.

Pharmacokinetic studies were conducted using rat plasma through LC-MS/MS. The pharmacokinetic studies unveiled noteworthy differences between FPT and FPT-LP cocrystal in various parameters like AUC, half life, steady state volume of distribution and the drug concentration in plasma. Importantly, the clearance rate was decreased that suggests increased bioavailability of the developed cocrystals. The % bioavaibility calculation of FPT and FPT-LP showed an increase in the bioavailability of FPT-LP to an extent of 88.88 % resulting in an enhancement of absolute bioavaibility of FPT from 18 % to 32 %. The relative crystallinity obtained from the PXRD studies for the prepared FPT-LP cocrystals was found to be 66.98 %. These cocrystals showed remarkable increase in bioavaibility inspite of the

crystalline nature. The increase in bioavaibility for FPT-LP can be co-related to a decrease in the dose to about one third (41.25 mg) in comparison to FPT.

The FPT-LP cocrystals were formulated into capsule dosage form after diligent selection of the type and concentration of the disintegrant. The % cumulative drug release was the highest in FPT-LP capsule (99.95 %), while the observed values were lower for FPT capsule (85.21 %), Physical Mixture (83.57 %) and marketed tablet formulation (88.26 %) at 60 min. The stability of the FPT-LP capsule was assessed by Dynamic Vapor Sorption (DVS) Analysis and Accelerated Stability Studies. DVS studies proved that FPT-LP is non-hygroscopic at 120% RH. Accelerated stability studies were carried out for 3 months according to ICH Q1A (R2) which determined that FPT-LP cocrystal and the developed capsule formulation is stable. The predicted shelf life of the formulated FPT-LP capsule is 2 years which is equivalent to the marketed tablet formulation. The storage condition was predicted as room temperature (15 to 25 ºC).

The IVIVC studies showed that the correlation between in vitro and in vivo data that is between percentage drug Release and Absorption Rate is a Level-A correlation. This leads to the conclusion that there was a point to point correlation between the in vitro and in vivo data sets with % similarity of 99.77 % as coefficient of regression.

## 10.2 Conclusion

The development of FPT-LP cocrystals using Solvent Evaporation Technique and Supercritical Fluid Extraction (SFE) has yielded promising outcomes, marked by a high yield and notable improvements in the solubility of FPT. This innovative approach has resulted in cocrystals demonstrating a remarkable enhancement in bioavailability, thereby enhancing the therapeutic efficacy of the drug. The formulation of a capsule dosage form further underscores the potential of these cocrystals, showcasing superior in vitro release characteristics and evidence of stability.

The success of these FPT-LP cocrystals is particularly noteworthy due to their ability to address challenges associated with the solubility of FPT, a critical factor in drug absorption and efficacy. The enhanced solubility achieved through cocrystallization contributes to improved bioavailability, allowing for a more potent and effective therapeutic response. The choice of a capsule dosage form is strategic, considering

its advantages in terms of retaining the integrity of the cocrystal lattice and drug delivery.

The comprehensive analytical and pharmacokinetic studies conducted on these cocrystals provide valuable insights into their molecular interactions and behavior. This knowledge is crucial for fine-tuning the cocrystal synthesis process and tailoring formulations to maximize therapeutic outcomes.

In conclusion, the FPT-LP cocrystals prepared using solvent evaporation technique and SFE represent a promising advancement in pharmaceutical development. The combination of enhanced solubility, improved bioavailability, and favorable in vitro release characteristics position these cocrystals as a potential breakthrough in crystal engineering for FPT. The analytical and pharmacokinetic studies pave the way for their further exploration and application in the development of innovative and effective pharmaceutical product with potentially lower dosage and side effects.

The work done has already been patented and under examination; Title: Novel Cocrystals of Fimasartan and Process Thereof; Patent Number: IN 202221043126, Date of Publication: 12-Jan-2024 (Annexure-II).

## 10.3 Contribution of Research work

### 10.3.1 Benefit to the Society

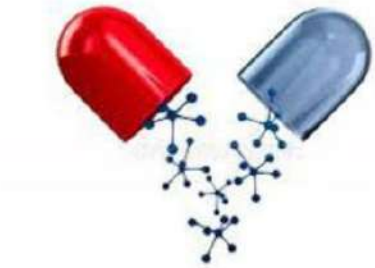
There are 1.2 billion people suffering from hypertension and being treated with antihypertensive molecules. The developed cocrystal of FPT-LP offers substantial advantages by significantly increasing the bioavailability of FPT. This leads to a remarkable increase from 18% to 32% that is 88% increase in bioavailability which is a substantial enhancement, indicating that a much larger portion of the administered FPT dose now reaches the systemic circulation. Advantages being offered would lead to lowering of the dose and increase in safety with desired efficacy.

### 10.3.2 Benefit to the Industry

The easy scalability of FPT-LP by SFE technique makes it suitable for large-scale production. Moreover, the comparison of FPT-LP with FPT highlights several beneficial changes, including lower dosing, simplified manufacturing processes, and potential cost savings in formulation. These advantages make FPT-LP a promising candidate for further development and pharmaceutical use, offering improved drug delivery and potential cost benefits to patients and healthcare providers alike.

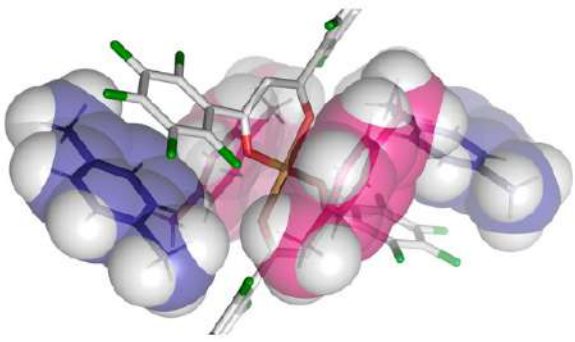
### 10.3.3 Regulatory Perspective

According to the United States Food and Drug Administration (USFDA), cocrystals of existing drugs have the potential to be patented as new cocrystal forms. The cocrystals are formed by the interactions between the API and coformer leading to the formation of a new crystalline lattice with distinct properties compared to the individual components. The bonding combination of FPT and L-Proline provides a platform for the establishment of inventive step. The research work is already protected by patent filling. Patent protection for cocrystals encourages pharmaceutical innovation, leading to the development of more effective and efficient drug formulations for the benefit of patients and healthcare providers.



# Chapter-11

# References



# CHAPTER - 11

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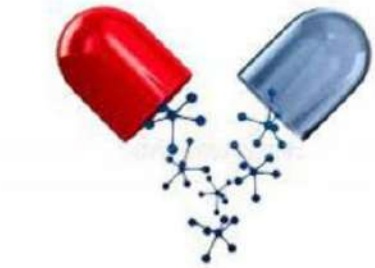
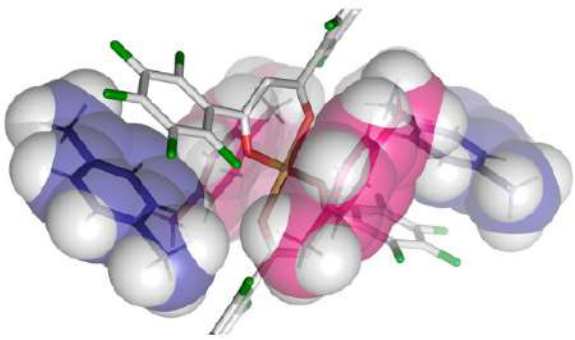


# Annexure

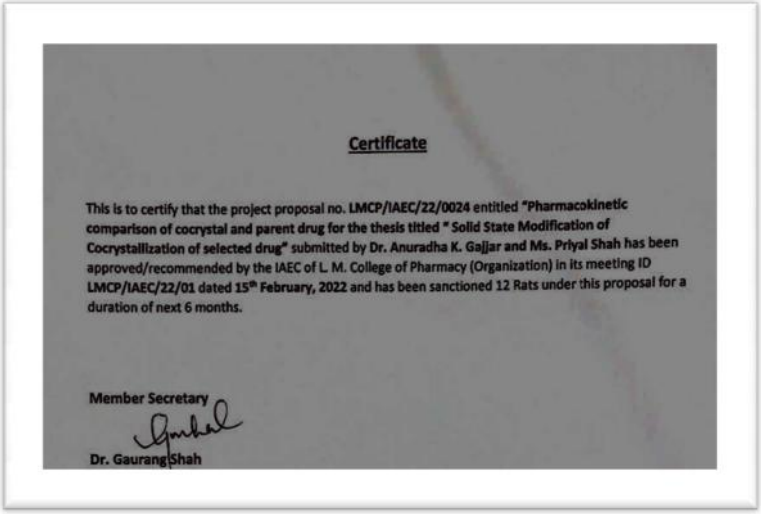


# Annexure

| | |
|---|---|
| Annexure I | IAEC Approval Letter |
| Annexure II | Patent Application |
| Annexure III | Fellowship Letter |
| Annexure IV | Summary of Compliance Report (DPC - I to DPC - VI) |
| Annexure V | List of Publications |
| Annexure VI | List of Conference Presentations |
| Annexure VII | List of Major Participations |

# ANNEXURE-I

## IAEC Approval Letter



**Certificate**

This is to certify that the project proposal no. **LMCP/IAEC/22/0024** entitled **"Pharmacokinetic comparison of cocrystal and parent drug for the thesis titled " Solid State Modification of Cocrystallization of selected drug"** submitted by **Dr. Anuradha K. Gajjar and Ms. Priyal Shah** has been approved/recommended by the IAEC of L. M. College of Pharmacy (Organization) in its meeting ID **LMCP/IAEC/22/01** dated **15th February, 2022** and has been sanctioned 12 Rats under this proposal for a duration of next 6 months.

**Member Secretary**

**Dr. Gaurang Shah**

# ANNEXURE-II

## Patent Application

| Patent Status | Provisional | Complete | Published |
|---|---|---|---|
| Patent No | 202221043126 | IN 202221043126 | 202221043126 A |
| Filling Date | 27-Jul-2022 | 13-Jul-2023 | 12-Jan-2024 |
| Title | Novel Cocrystals of Fimasartan and Process Thereof | | |

8/13/22, 3:45 PM ipindiaservices.gov.in/Verify/chkCert.aspx?prm1=FzcJYOYg6oleykt7gV5SlQ==&prm2=M68T3PSq5br6NFGkzqILWQ==&prm...

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते

**Application Filing Receipt**

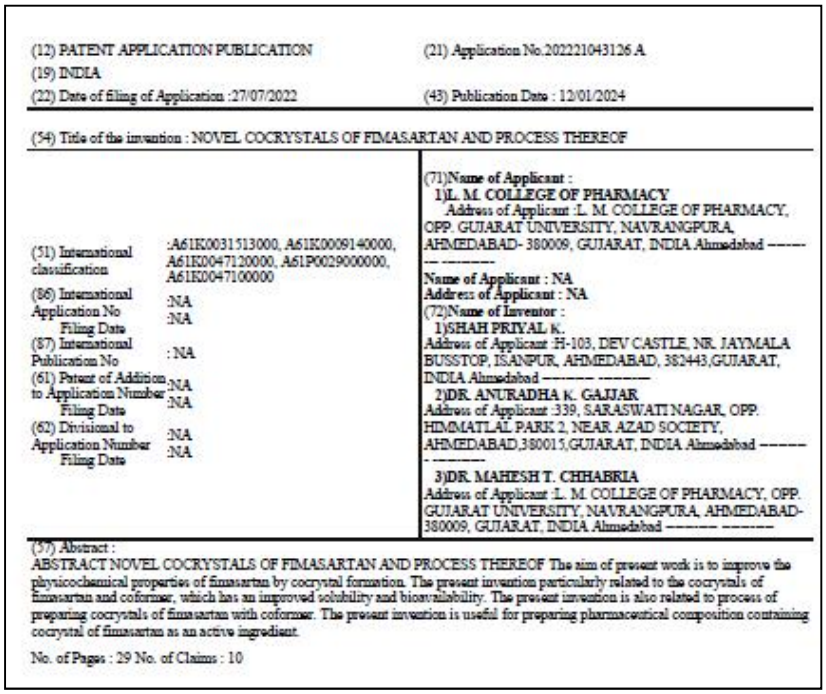
**Goverment of India**
**Patent Office**
Intellectual Property Office Building,
S.M. Road, Antop Hill,
Mumbai-400037
Phone- 022-24137701,24142026
Fax: 022-24130387
e-mail: mumbai-patent@nic.in

**CBR Number : 19376** **CBR date: 27-07-2022**

Application Type: ORDINARY APPLICATION
Priority Number:
Priority Date:
Priority Country: Not Selected

To,
L. M. COLLEGE OF PHARMACY
EXCELON IP - 627, Gala Empire, Drive In Road, Thaltej, Ahmedabad, Gujarat 380052, INDIA.

Received documents purporting be to an application for patent numbered 202221043126 dated 27-07-2022 by L. M. COLLEGE OF PHARMACY of L. M. COLLEGE OF PHARMACY, OPP. GUJARAT UNIVERSITY, NAVRANGPURA, AHMEDABAD- 380009, GUJARAT, INDIA relating to NOVEL COCRYSTALS OF FIMASARTAN AND PROCESS THEREOF together with the Provisional and fee(s) of ₹1600 ( One Thousand Six Hundred only).



(12) PATENT APPLICATION PUBLICATION
(19) INDIA
(22) Date of filing of Application :27/07/2022

(21) Application No.202221043126 A
(43) Publication Date : 12/01/2024

(54) Title of the invention : NOVEL COCRYSTALS OF FIMASARTAN AND PROCESS THEREOF

(51) International classification :A61K0031513000, A61K0009140000, A61K0047120000, A61P0029000000, A61K0047100000
(86) International Application No :NA
Filing Date :NA
(87) International Publication No : NA
(61) Patent of Addition to Application Number :NA
Filing Date :NA
(62) Divisional to Application Number :NA
Filing Date :NA

(71)Name of Applicant :
1)L. M. COLLEGE OF PHARMACY
Address of Applicant :L. M. COLLEGE OF PHARMACY, OPP. GUJARAT UNIVERSITY, NAVRANGPURA, AHMEDABAD- 380009, GUJARAT, INDIA Ahmedabad ------ --- --------
Name of Applicant : NA
Address of Applicant : NA
(72)Name of Inventor :
1)SHAH PRIYAL K.
Address of Applicant :H-103, DEV CASTLE, NR. JAYMALA BUSSTOP, ISANPUR, AHMEDABAD, 382443,GUJARAT, INDIA Ahmedabad -------- --------
2)DR. ANURADHA K. GAJJAR
Address of Applicant :339, SARASWATI NAGAR, OPP. HIMMATLAL PARK 2, NEAR AZAD SOCIETY, AHMEDABAD,380015,GUJARAT, INDIA Ahmedabad ------ - --------
3)DR. MAHESH T. CHHABRIA
Address of Applicant :L. M. COLLEGE OF PHARMACY, OPP. GUJARAT UNIVERSITY, NAVRANGPURA, AHMEDABAD-380009, GUJARAT, INDIA Ahmedabad -------- --------

(57) Abstract :
ABSTRACT NOVEL COCRYSTALS OF FIMASARTAN AND PROCESS THEREOF The aim of present work is to improve the physicochemical properties of fimasartan by cocrystal formation. The present invention particularly related to the cocrystals of fimasartan and coformer, which has an improved solubility and bioavailability. The present invention is also related to process of preparing cocrystals of fimasartan with coformer. The present invention is useful for preparing pharmaceutical composition containing cocrystal of fimasartan as an active ingredient.

No. of Pages : 29 No. of Claims : 10

## ANNEXURE -III

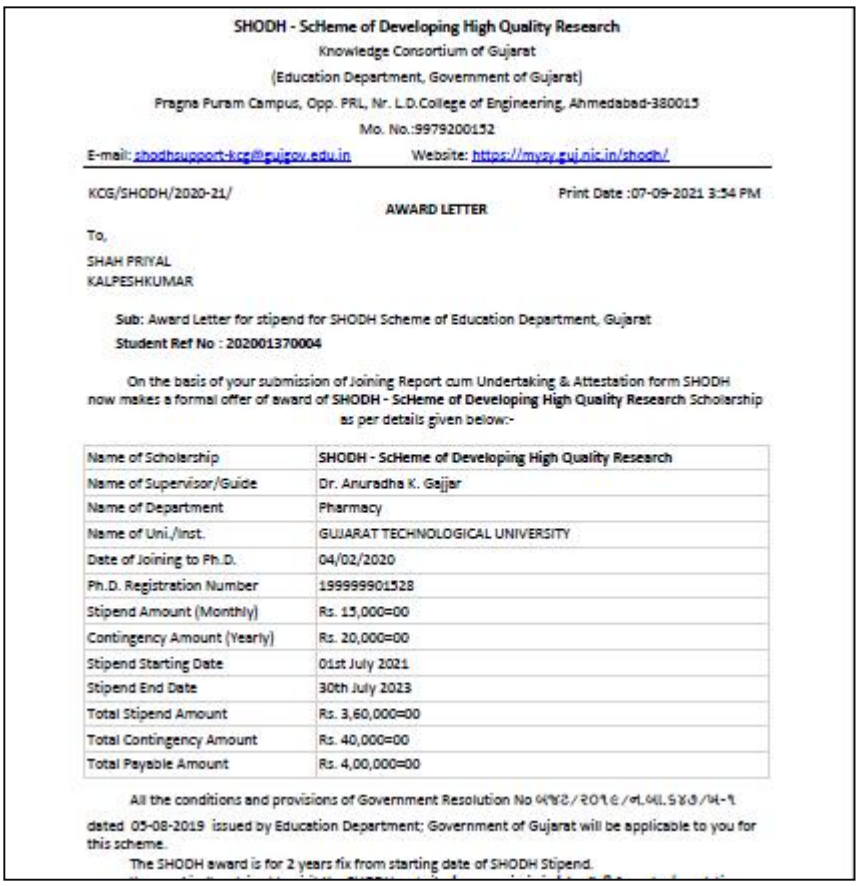
### Fellowship Letter

The SHODH (ScHeme of Developing High Quality Research) Fellowship was awarded by the Education Department, Government of Gujarat.

The aim of this fellowship is to increase orientation amongst the students and to develop high quality research.

The fellowship included stipend for two years and annual contingency.

**Tenure:** 01-Jul-2021 to 30-Jul-2023



**SHODH - ScHeme of Developing High Quality Research**
Knowledge Consortium of Gujarat
(Education Department, Government of Gujarat)
Pragna Puram Campus, Opp. PRL, Nr. L.D.College of Engineering, Ahmedabad-380015
Mo. No.:9979200152
E-mail: shodhsupport-kcg@gujgov.edu.in Website: https://myny.guj.nic.in/shodh/

KCG/SHODH/2020-21/ Print Date :07-09-2021 3:54 PM

**AWARD LETTER**

To,
SHAH PRIYAL
KALPESHKUMAR

**Sub:** Award Letter for stipend for SHODH Scheme of Education Department, Gujarat
**Student Ref No : 202001370004**

On the basis of your submission of Joining Report cum Undertaking & Attestation form SHODH now makes a formal offer of award of **SHODH - ScHeme of Developing High Quality Research** Scholarship as per details given below:-

| | |
|---|---|
| Name of Scholarship | **SHODH - ScHeme of Developing High Quality Research** |
| Name of Supervisor/Guide | Dr. Anuradha K. Gajjar |
| Name of Department | Pharmacy |
| Name of Uni./Inst. | GUJARAT TECHNOLOGICAL UNIVERSITY |
| Date of Joining to Ph.D. | 04/02/2020 |
| Ph.D. Registration Number | 199999901528 |
| Stipend Amount (Monthly) | Rs. 15,000=00 |
| Contingency Amount (Yearly) | Rs. 20,000=00 |
| Stipend Starting Date | 01st July 2021 |
| Stipend End Date | 30th July 2023 |
| Total Stipend Amount | Rs. 3,60,000=00 |
| Total Contingency Amount | Rs. 40,000=00 |
| Total Payable Amount | Rs. 4,00,000=00 |

All the conditions and provisions of Government Resolution No [illegible] dated 05-08-2019 issued by Education Department; Government of Gujarat will be applicable to you for this scheme.

The SHODH award is for 2 years fix from starting date of SHODH Stipend.

## ANNEXURE –IV

### Summary of DPC Compliance Report (DPC-I to DPC-VI)

| Sr. No | Date | Comments |
|---|---|---|
| DPC – I | 26-Dec-2020 | Preliminarily explore the reported co-formers and techniques for the cocrystal formation. |
| DPC – II | 05-Jun-2021 | LOQ Calculation needs to be checked.<br>Use various solvents in wet grinding method.<br>Solubility and physical evaluation of promising cocrystals should be studied. |
| DPC – III | 23-Oct-2021 | Explore the study of Ph and pKa to predict cocrystal formation.<br>Study the properties essential for tableting for the optimized cocrystals. |
| DPC-IV | 22-Apr-2022 | Good Publication. |
| DPC-V | 07-Oct-2022 | Detailed interpretation of the pharmacokinetic studies.<br>Appropriate formulation to be prepared and stability studies. |
| DPC-VI | 24-Apr-2023 | Appreciated the outcome of the project and Recommended for Open Seminar. |

# ANNEXURE-V

## List of Publications

### Publication-1

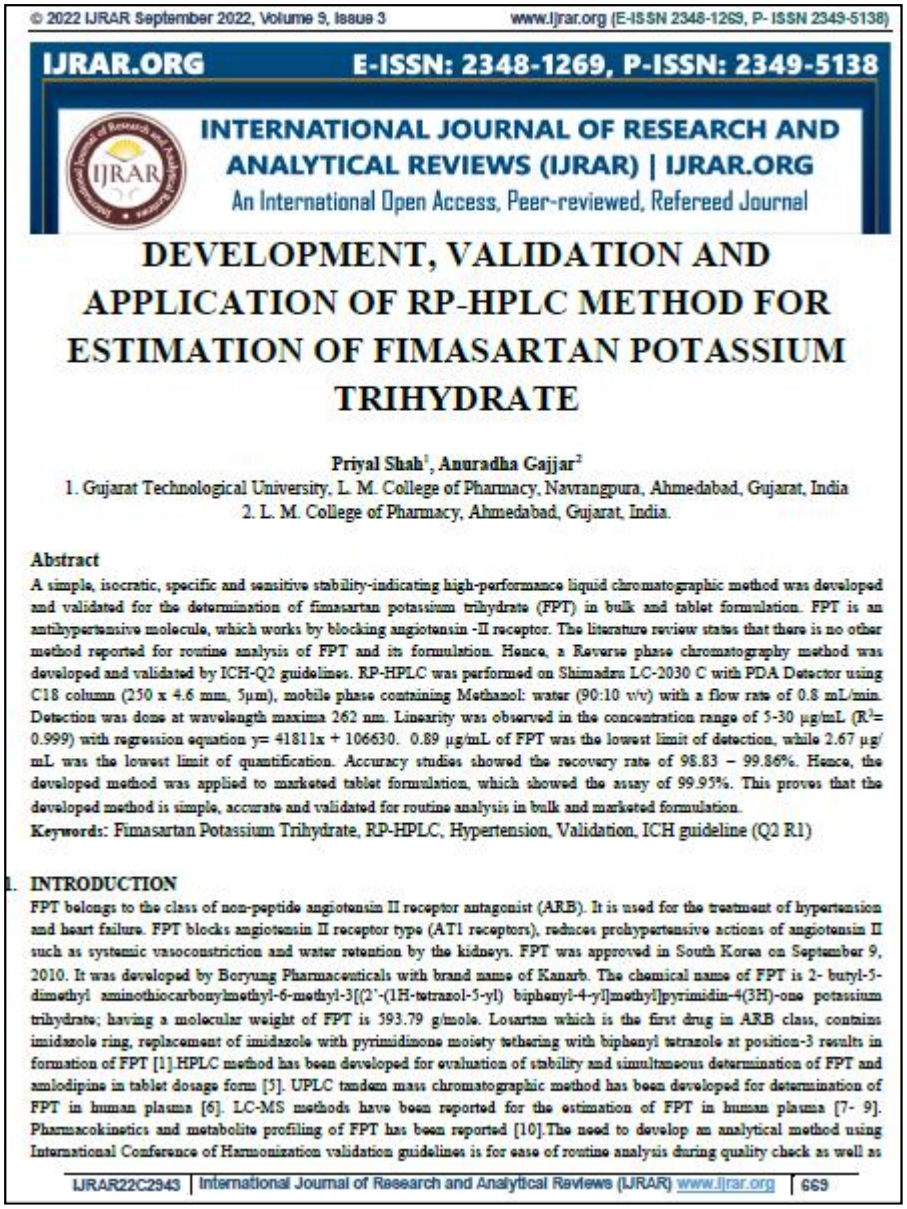
**Type of Publication:** Research

**Title:** Development, Validation and Application of RP-HPLC Method for estimation of Fimasartan Potassium Trihydrate

**Journal:** International Journal of Research and Analytical Reviews (IJRAR)

**Published in:** Volume 9 | Issue 3 | September 2022, 669-675

**UGC Approved Journal No:** 43602



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IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

# DEVELOPMENT, VALIDATION AND APPLICATION OF RP-HPLC METHOD FOR ESTIMATION OF FIMASARTAN POTASSIUM TRIHYDRATE

**Priyal Shah[1], Anuradha Gajjar[2]**

1. Gujarat Technological University, L. M. College of Pharmacy, Navrangpura, Ahmedabad, Gujarat, India

2. L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

**Abstract**

A simple, isocratic, specific and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of fimasartan potassium trihydrate (FPT) in bulk and tablet formulation. FPT is an antihypertensive molecule, which works by blocking angiotensin -II receptor. The literature review states that there is no other method reported for routine analysis of FPT and its formulation. Hence, a Reverse phase chromatography method was developed and validated by ICH-Q2 guidelines. RP-HPLC was performed on Shimadzu LC-2030 C with PDA Detector using C18 column (250 x 4.6 mm, 5µm), mobile phase containing Methanol: water (90:10 v/v) with a flow rate of 0.8 mL/min. Detection was done at wavelength maxima 262 nm. Linearity was observed in the concentration range of 5-30 µg/mL ($R^2$= 0.999) with regression equation y= 41811x + 106630. 0.89 µg/mL of FPT was the lowest limit of detection, while 2.67 µg/mL was the lowest limit of quantification. Accuracy studies showed the recovery rate of 98.83 – 99.86%. Hence, the developed method was applied to marketed tablet formulation, which showed the assay of 99.95%. This proves that the developed method is simple, accurate and validated for routine analysis in bulk and marketed formulation.

**Keywords:** Fimasartan Potassium Trihydrate, RP-HPLC, Hypertension, Validation, ICH guideline (Q2 R1)

## 1. INTRODUCTION

FPT belongs to the class of non-peptide angiotensin II receptor antagonist (ARB). It is used for the treatment of hypertension and heart failure. FPT blocks angiotensin II receptor type (AT1 receptors), reduces prohypertensive actions of angiotensin II such as systemic vasoconstriction and water retention by the kidneys. FPT was approved in South Korea on September 9, 2010. It was developed by Boryung Pharmaceuticals with brand name of Kanarb. The chemical name of FPT is 2- butyl-5-dimethyl aminothiocarbonylmethyl-6-methyl-3[(2'-(1H-tetrazol-5-yl) biphenyl-4-yl]methyl]pyrimidin-4(3H)-one potassium trihydrate; having a molecular weight of FPT is 593.79 g/mole. Losartan which is the first drug in ARB class, contains imidazole ring, replacement of imidazole with pyrimidinone moiety tethering with biphenyl tetrazole at position-3 results in formation of FPT [1].HPLC method has been developed for evaluation of stability and simultaneous determination of FPT and amlodipine in tablet dosage form [5]. UPLC tandem mass chromatographic method has been developed for determination of FPT in human plasma [6]. LC-MS methods have been reported for the estimation of FPT in human plasma [7- 9]. Pharmacokinetics and metabolite profiling of FPT has been reported [10].The need to develop an analytical method using International Conference of Harmonization validation guidelines is for ease of routine analysis during quality check as well as

## Publication-2

**Type of Publication:** Research

**Title:** Development and Validation of Stability Indicating RP-HPLC Method for Efonidipine Hydrochloride Ethanolate

**Journal:** International Journal of Innovative Research in Technology (IJIRT)

**Published in:** Volume 8 | Issue 6 | November 2021, 340-345

**UGC Approved Journal No:** 47859

© November 2021| IJIRT | Volume 8 Issue 6 | ISSN: 2349-6002

# Development and Validation of Stability Indicating RP-HPLC Method for Efonidipine Hydrochloride ethanolate

Ms. Priyal Shah[1], Dr. Anuradha Gajjar[2]

*1. Research Scholar, Gujarat Technological University, L.M. College of Pharmacy, Ahmedabad*
*2. Head of the Department of Pharmaceutical Chemistry and Quality Assurance, L. M. College of Pharmacy, Ahmedabad*

*Abstract* - **The paper describes development and validation of a stability indicating chromatographic assay method for Efonidipine Hydrochloride ethanolate (EFD) in solid pharmaceutical dosage form. EFD and degradant products under varied stress conditions like hydrolysis at a range of pH, temperature, oxidation and exposure to light were analysed by developed RP-HPLC method with proper separation as well as good peak shape. The developed method consisted of mobile phase Methanol: water (50:50 v/v). The flow rate was 0.8 mL/min with a run time of 10 min and detection at 270 nm. The assay was performed on marketed formulation that showed 99.52% labelled claim. During the forced degradation studies, Efonidipine showed maximum degradation (10 %) under oxidative stress followed by 8 % photodegradation. The drug showed lower degradation under acid, base and thermal stress conditions, to the extent of 4 %, 3 % and 6 % respectively. It was also observed that the retention time of the degradant under photolytic and oxidative degradation were the same probably due to the formation of the same product.**

*Index Terms* - **Efonidipine hydrochloride ethanolate, Stability indicating assay method, stress conditions, anti-hypertensive, photodegradation.**

### 1.1 INTRODUCTION

Efonidipine hydrochloride ethanolate (EFD), (±)-2-[Benzyl(phenyl) amino] ethyl-1,4-dihydro-2,6-dimethyl-5-(5,5-dimethy-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitorophenyl)-3-dihydropyridine derivatives are subject to the first-pass effect, and the primary metabolism step involves oxidation of the dihydropyridine ring to the corresponding pyridine analogue [5,6]. However, it has been suggested that EFD is less likely to undergo the first-pass effect and its dihydropyridine ring is oxidized mainly after metabolism of the side chain [7]. Additionally, EFD has distinct properties when compared with other calcium channel blockers. The objective of the study was to develop a specific, simple, rapid, reliable and validated stability indicating assay method for the estimation of EFD in accordance with International Conference on Harmonization (ICH) guidelines.

### 1.2 MATERIAL AND METHODS

#### 1.2.1 Reagents and Materials

Efonidipine Hydrochloride Ethanolate (EFD, 99.0% pure) was received as a gift sample from Ajanta Pharma Ltd. (Mumbai, India). HPLC-grade Methanol was purchased from Merck, India.AR grade HCl, NaOH pellets and $H_2O_2$ was obtained from SD Fine Chem Ltd. The deionized and ultra-pure water used in all experiments was obtained from the Milli-Q System (Millipore).

#### 1.2.2 Instrumentation and chromatographic conditions

LC was performed with Shimadzu equipment LC 2010 that was equipped with auto sampler, UV visible

## Other Publications

1. Jalpa Suthar, **Priyal Shah**, Krupali Patel, Pankti Pathak "A Study on Drug Utilization in Hypertension in Medical Care Hospital", *Indian Journal of Public Health Research and Development*,2020, Vol:11, Issue:03, ISSN 0976-5506 (Accepted)

2. Manan Patel, Romansha Beri, **Priyal Shah**, "Nasal Drug Delivery System and it's Application", *International Journal of Research and Analytical Reviews*, 2021, Vol:08, Issue:02, ISSN 2348-1269 (Accepted)

3. Manan Patel, Nirav Shah, Dhruvi Dave, **Priyal Shah**, "A Review on Effectivity of Plant Based Vaccines in The Treatment of Viral Disease", *Journal of Drug Delivery and Therapeutics*, 2021, Vol:11, Issue:03, ISSN 2250-1177 (Accepted)

4. **Priyal Shah**, Anuradha Gajjar, "A promising approach for tailoring properties of API by Crystal Engineering" Communicated to *Journal of Pharmaceutical Sciences*. Current Status: Under Review

## ANNEXURE-VI

## List of Conference Presentations

### Poster Presentation-1

Conference: Prof. ANM Memorial International Conference

Theme: Recent Advances and Trends in NDDS

Organised by: Maharaja Sayajirao University, Vadodara

Date: 23-25 Sep 2021

**Title:** Crystal Engineering: An Emerging Tool to Augment Solubility

CERTIFICATE

This certificate is presented to
PRIYAL KALPESHKUMAR SHAH (Co-author/s: Anuradha Gajjar)
for participation in E-Poster competition held on 23rd Sept 2021 at

Prof. Ambikanandan Misra Memorial International E-Conference on
"Recent Advances & Trends in Novel Drug Delivery Systems" 23rd - 25th September, 2021

*Organized by*
MSU Pharmacy Alumni Association (MPA) &
Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda

Dr. Prashant Mahumkar, Secretary MPA | Dr. Jayan I.S. Devi, President MPA | Prof. Krutika Sawant, Dean, Faculty of Pharmacy

### Poster Presentation-2

Conference: GUJCOST sponsored International e-conference

Theme: Vital Role of Polymers in Drug Delivery

Organised by: L. J. University, Ahmedabad

Date: 13-14 Aug 2021

**Title:** Investigating Polymers as Coformers in Design of Cocrystals

LJ University

GUJCOST Sponsored Two-days International e-Conference on

'VITAL ROLE OF POLYMERS IN DRUG DELIVERY'

CERTIFICATE OF PARTICIPATION

THIS IS TO CERTIFY THAT

PRIYAL SHAH

Participated in e-Presentation

in

GUJCOST sponsored International e-Conference on 'Vital Role of Polymers in Drug delivery' organized by L. J. Institute of Pharmacy, L. J. University, Ahmedabad on 13th and 14th August, 2021

MR. JAYMIN PATEL | DR. SHREERAJ SHAH | MS. KAUSHIKA PATEL

**Poster Presentation-3**

Conference: Pharma Anveshan-2023

Theme: Pharmacy

Organised by: Pharmacy Council of India, Government of India, New Delhi

Date: 06-Mar-2023

**Title:** Augmenting Bioavailability by Crystal Engineering

## ANNEXURE-VII

## LIST OF MAJOR PARTICIPATIONS

| Date | Days | Title | Organized by |
|---|---|---|---|
| 15-21 Mar 2023 | 07 | Workshop on Single Crystal X-ray Crystallography | IIT Gandhinagar |
| 12 May 2022 | 01 | Seminar Series on Crystal Engineering | ICT Mumbai |
| 07-11 Mar 2022 | 05 | Workshop on Transitional Research – Taking Novel Drugs from Lab to Clinic | ISCR Mumbai |
| 24-26 Feb 2022 | 03 | International Symposium ($20^{th}$) on Advances in Technology and Business Potential of NDDS | Controlled Release Society (CRS) |
| 17-22 Jan 2022 | 07 | New Era Technologies in Formulation Design Space | Panjab University, Chandigarh |
| 05 Aug 2021 | 01 | Cocrystal: A Promising Approach for Tailoring Properties of API | Kamla Nehru College of Pharmacy, Nagpur |
| 24-26 Jun 2021 | 03 | $10^{th}$ International Conference Disso India 2021 | SPDS and AAPS |
| 09-13 Aug 2021 | 05 | IPR short term training Program | GUSEC |
| 01 May 2020 | 01 | Research: Planning and Execution | D Y Patil, University Pune |

IIT – Indian Institute of Technology
ICT – Institute of Chemical Technology
ISCR – Indian Society for Clinical Research
SPDS- Society for Pharmaceutical Dissolution Science
AAPS- American Association of Pharmaceutical Scientists
GUSEC- Gujarat University Startup and Entrepreneurship Council