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Scientific Coformer Screening, Preparation and Evaluation of Fenofibrate Tartaric Acid Cocrystal

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ABSTRACT

Objective: This present study aims to screen pharmaceutical cocrystal of Fenofibrate and coformers. Further the preparation and evaluation of fenofibrate-coformer cocrystal and *In-Vitro* drug release and *Ex-Vivo* Permeation study was done. **Material and Methods:** The coformers for Fenofibrate were screened using molecular docking. The cocrystals produced were characterized using Differential Scanning Calorimetry (DSC), X-ray diffraction (XRPD) study and Infrared spectroscopy. **Results:** Cocrystal of Fenofibrate with tartaric acid was successfully prepared. The cocrystals displayed enhanced dissolution rate by 2.36 fold, similarly the *ex-vivo* drug uptake through everted chicken intestine model was improved by 4.38 fold. The formation of cocrystals of fenofibrate with tartaric acid was evaluated by DSC, IR and XRPD.

Conclusion: The fenofibrate - tartaric acid cocrystal exhibited increased % drug release and permeation compared to fenofibrate. This study confirms that selection of proper coformer is very vital step in preparation of stable, superior cocrystal. Based upon above study and results it revealed that cocrystallization offers a valuable way to improve the physicochemical properties of the API.

Keywords: Pharmaceutical Cocrystal, Fenofibrate , Coformer, Molecular docking.

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INTRODUCTION

Pharmaceutical cocrystallization is a reliable method to modify and improve physical and chemical properties of drugs such as solubility, stability, dissolution rate, hygroscopicity and compressibility without changing their pharmacological activity.[1] Pharmaceutical cocrystals offer an alternative to chemical modification of the drug substance as well as established salt, solvate, amorphous, inclusion complexes and polymorphic drug forms all of which have restrictions in their utility. Formation of cocrystal depends on the functional groups between API and coformer, to allow for the occurrence of hydrogen bonds or other forms of solid interaction.[2] Cocrystals consist of two or more neutral molecular components in a crystal lattice with defined stoichiometry. These are homogeneous phases, which are solids at room temperature and are held together by weak interactions, mainly hydrogen bonding.

Capability of forming non-covalent interactions especially hydrogen bonds with an APIs forms the basis of coformer selection. Various approaches to coformer selection were supramolecular synthon approach, Hansen solubility parameter, pKa based, lattice energy calculation, hydrogen bond propensity and Molecular docking.[3]

Fenofibrate is a extensively used as hypolipidemic drug. This drug is used mostly in lipid regulation as it decreases low-density lipoprotein (LDL) and very-low density lipoprotein (VLDL) levels, and increases high density lipoprotein (HDL) level. Solubility and permeability are the fundamental parameters controlling the rate and extent of drug absorption. Fenofibrate is a BCS class II drug with low solubility and high permeability. Various reported methods to improve dissolution of fenofibrate are micronization, nanonization, salt formation, incorporation of surface active agent, solid dispersion, polymorphism and cocyrstal synthesis. [4,5,6,7]

The aim of the study was to improve dissolution of Fenofibrate using cocrystal formation. The study involved *in silico* screening of coformers, preparation and *in-vitro, ex-vivo* evaluation of Fenofibrate cocrystals. The anti-solvent addition method was used to form cocrystals with tartaric acid. The cocrystals were characterized by IR spectroscopy, DSC and XRPD.

MATERIALS AND METHODS

Materials

Fenofibrate was provided by Medley pharmaceutical Ltd. Coformers catechol, tartaric acid, anthranillic acid and ferullic acid were purchased from LOBA CHEMIE PVT LTD. All required solvents and excipients were provided by LOBA CHEMIE PVT LTD . Molecular docking study was performed using Schrodinger suite version 9.0 software.

Methods

Selection of coformers

Molecular Docking

The coformers were initially selected based upon supramolecular synthon approach that depicts possibility of hydrogen bond formation with fenofibrate[1].The structures of four coformers; catechol (Chem Spider ID: 13837760), tartaric acid (Chem Spider ID:852), anthranillic acid (Chem Spider ID:222) and ferulic acid (Chem Spider ID: 393368) were retrieved from chemspider database .

Coformer structures were prepared by LigPrep 2.3 module of Schrodinger suite [8]. The structure of fenofibrate was prepared using protein preparation wizard of Maestro [9]. The protrin structure was optimized and minimized using OPLS-2005 force field. Molecular docking was performed using Glide docking program [10]. The results were run on the basis of glide score.

Preparation of fenofibrate cocrystals:

1) Antisolvent addition method [1,3]

Fenofibrate and coformer weigh in 1:1 molar ratios were dissolved in 25 ml ethanol using moderate stirring. The solution was then filtered through a Whatman filter paper to remove any undissolved material. Distilled water was then added dropwise to the above solution with constant stirring to induce cocrystal precipitation. The cocrystals were allowed to dry overnight in desiccators.

Evaluation of Cocrystal formation :

The prepared cocrystal in present study was primarily confirmed by comparing DSC results, IR results and XRD study of cocrystals with fenofibrate (pure drug) with selected coformers. *In vitro* dissolution and *ex-vivo* permeation studies were carried out.

Standard Calibration Curve of Fenofibrate

Standard calibration curve of fenofibrate was developed by suitably diluting methanolic stock solution of fenofibrate in 0.5% SLS solution in distilled water to obtain concentrations between 2 to 10µg/ml. The absorbance of resulting solutions was measured at 286 nm using double beam UV-Visible Spectrophotometer against 0.5 % SLS solution as blank.

Differential Scanning Calorimetry (DSC):

DSC was performed on Mettler-Toledo DSC 823* (Columbus) instrument and an empty standard aluminium pan were used as reference. DSC scans were recorded at heating rate of 10 °C/ min in temperature range 30-300 °C, DSC measurements were carried out on prepared fenofibrate cocrystal.

X-ray diffraction (XRPD) study:

Powder X-ray diffraction pattern of pure fenofibrate and fenofibrate cocrystal was investigated using powder X-ray diffractometer(PW 1729 X-ray Generator, Philips Ltd.). The X-rays were Ni filtered CuKα1 radiation with 40 KV and 30 mA over 0-100°/2θ.

Infrared spectroscopy:

IR (Brucker,Germany) was used for collecting the IR samples. The spectra were collected over the range of 4000-600 cm^{-1} in 32 scans, with resolution of 4 cm^{-1} for each sample.

Determination of drug content:

Drug content was determined by dissolving samples of co-crystals equivalent to 10 mg fenofibrate in 100 ml of methanol. After suitable dilution absorbance of resulting solutions was measured at 286 nm using double beam UV-Visible Spectrophotometer.

In- vitro dissolution study: [10]

In vitro dissolution study was carried out using USP dissolution apparatus II.The rotation speed of the paddles was set to 100 rpm. About 900 mL of 0.5 % Sodium lauryl sulphate (SLS) at 37 ± 0.5°C was used as the dissolution medium. At predetermined time intervals 5 mL samples were withdrawn, filtered through 0.45 µm membrane immediately, and 5 mL blank dissolution medium was added for replenishing of the dissolution medium, respectively. The amount of dissolved drug was determined at 286 nm using a UV spectrophotometer.

Ex-vivo permeation studies using everted chicken intestine [11]

To understand the absorption mechanism of fenofibrate and fenofibrate cocrystal, everted gut sac studies using chicken intestinal segments were performed. Intestine was washed carefully with Krebs ringer solution and different segments of small intestine were identified. A length of 8–10 cm was rapidly removed and gently everted over a glass rod. The everted intestine was then slipped off the glass rod and placed in a flat dish containing Krebs–Henseleit bicarbonate (KHB) buffer oxygenated with O_2/CO_2 (95%/5%) at 37°C. The *in vitro* absorption system consisted of USP dissolution apparatus II operated at 100 rpm containing 5% SLS (900 ml) as dissolution medium maintained at 37±0.5°C. Modified perfusion apparatus hol ding isolated everted intestine segment was placed in dissolution vessel. In this system, drug dissolution from formulation and permeation across everted intestine occurred simultaneously.

The fenofibrate and fenofibrate cocrystals was transferred in separate dissolution vessels. The aliquotes were collected at predetermined time intervals of 5, 10, 15, 30, 45, 60 min and the equal volumes of dissolution and serosal fluids were replaced. The samples were analyzed spectrophotometrically at 286 nm.

RESULTS AND DISCUSSION

Molecular Docking

The molecule of fenofibrate structure consists of two aromatic rings , two carbonyl groups, ether group and chlorine group. Fenofibrate molecule has three hydrogen bond acceptors as well as seven rotatable bond count., hence it can easily form co-crystals with other co-formers(figure 1). Coformers chosen in this work were catchol, tartaric acid, anthranillic acid and ferullic acid . The result of virtual screening of conformers using molecular docking is shown in table 1.

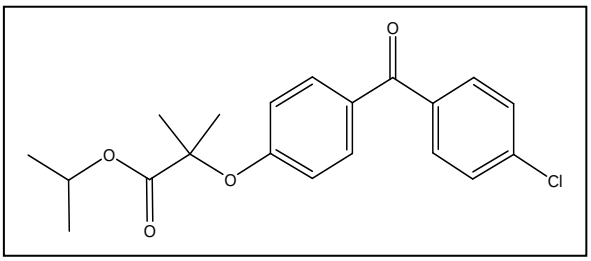
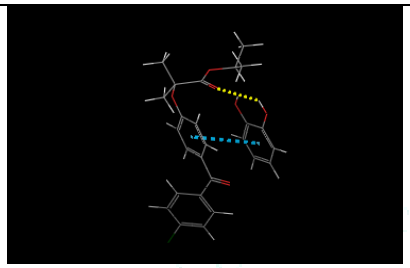
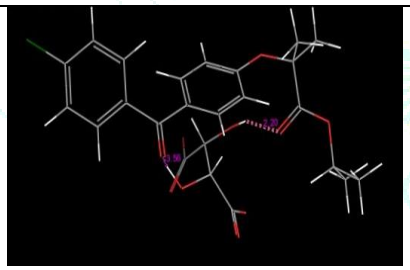
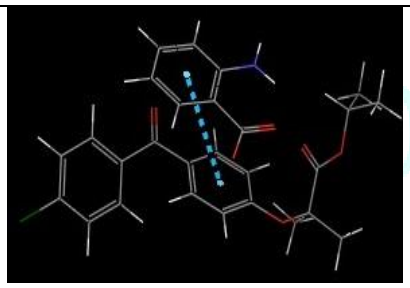
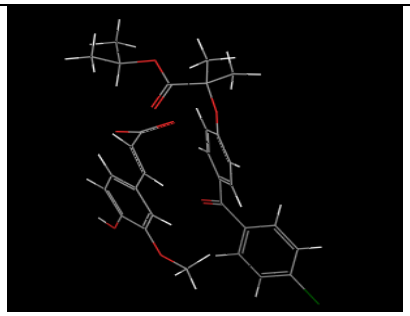


Figure-1 Molecular structure of fenofibrate

Glide score and hydrogen bonding parameters were used to determine the best possible coformer. The lowest value of Glide score shows the best interaction between fenofibrate and the coformer. The maximum hydrogen bond formation assists in higher binding affinity. The lowest Glide score of -4.28 kcal/mol and highest number of hydrogen bond formation with fenofibrate was exhibited by tartaric acid. Therefore, on the basis of number of hydrogen bond and least glide score tartaric acid coformer was selected for further studies.

Table 1- Virtual screening of co-formers with fenofibrate using molecular docking

Coformer	Structure	Type of Interaction	Fenofibrate atoms involved in binding	Coformer atoms involved in binding	Glide-score
Catechol		1)Hydrogen bonding. 2)π-π interaction	-C=O	H (-OH)	-1.38
tartaric acid		1)Hydrogen bonding.	-C=O	H (-OH)	-4.28
anthranillic acid		1) π-π interaction	--	--	-0.14
Ferulic acid		No interaction	--	--	--

Standard Calibration Curve of fenofibrate:

The absorption maxima of fenofibrate are reported at 286. nm. A linear relationship between the concentration and absorbance of fenofibrate were established over the examined concentration range (2-10 µg/mL). Calibration curve of fenofibrate was established. The equation of line was y = 0.056x + 0.027 and R^2 = 0.997 (figure-2)

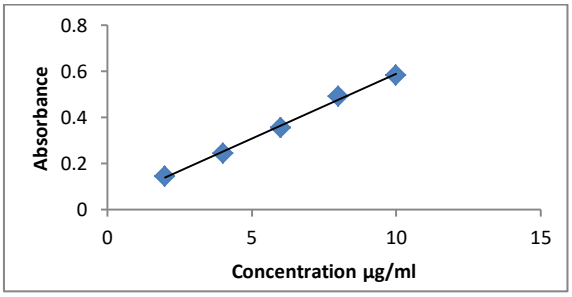


Figure-2: Calibration curve of fenofibrate

Differential Scanning Calorimetry (DSC)

DSC was conducted to investigate the molecular state of fenofibrate into cocrystal. DSC thermograms obtained for fenofibrate(Figure -3) and Finofibrate tartaric acid cocrystal (Figure -4) showed well-defined endothermic peak at 81.52°C corresponding to the melting point of crystalline drug and prepared cocrystals showed endothermic peaks at 83.67°C . This is due to increased crystallinity observed fenofibrate showed 316% crystallinity while fenofibrate tartaric acid cocrystal showed 560.90% Crystallinity.

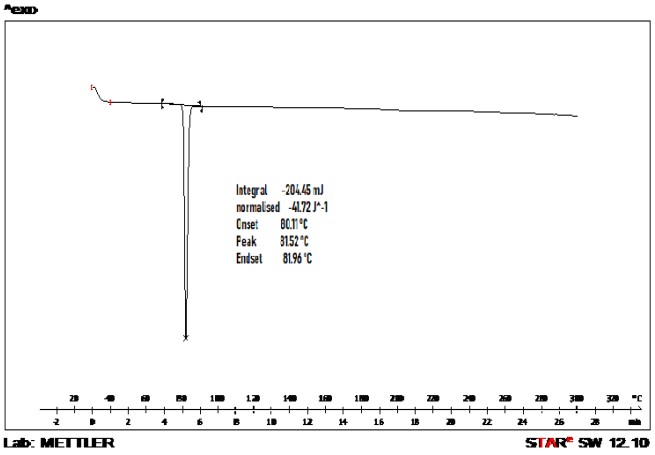


Figure -3: DSC thermograph of fenofibrate

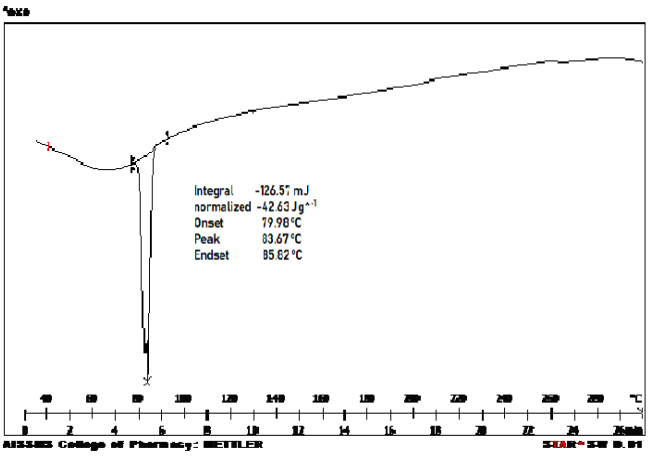


Figure -4: DSC thermograph of fenofibrate-tartaric acid cocrystal

Infrared spectroscopy:

The possible interaction between the drug and the coformers was studied by IR spectroscopy. From the results of IR, it was observed that all the important peaks due to functional groups of fenofibrate were present, the peak at 2982.49cm -1 indicates aromatic C-H stretching, peak at 1590cm -1 indicates C=O stretching, whereas peaks at 1287cm -1 and 1093cm -1 indicate aralkyl and dialkyl ether C-O stretching, respectively(figure-5). Tartaric acid IR showed a characteristic peak OH at 3352.53 cm-1 and C=O peak at 1708.96 cm $^{-1}$.

However some changes in the cocrystal IR spectrum were observed such as presence of peak at 1737.43 cm $^{-1}$ and OH stretch at 3260.64 cm $^{-1}$ in prepared fenofibrate cocrystals (figure-6) when compared to pure drug, thereby indicating that hydrogen bonding has occurred in the cocrystals .

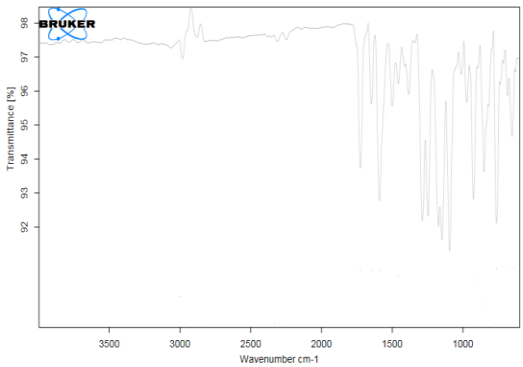


Figure -5: IR fenofibrate

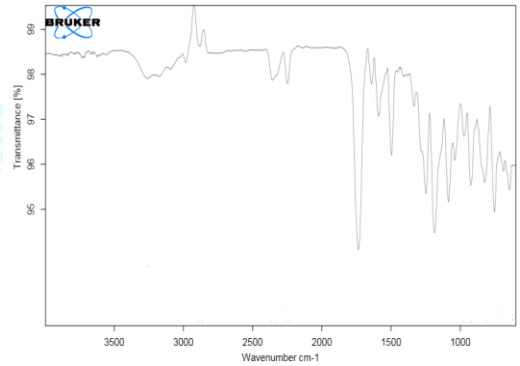


Figure -6: IR fenofibrate-tartaric acid cocrystal

X-ray diffraction (XRPD) study:

The formation of cocrystal was confirmed on the basis of XRPD studies which showed differences in diffractogram of cocrystal and fenofibrate. The diffraction pattern of cocrystal is completely different from fenofibrate and fenofibrate tartaric acid cocrystal showed characteristic diffraction peaks at different 2θ values (14.71, 16.52, 16.42, 22.53) (Figure-7)and 2θ values (12.31, 21.31, 22.73, 25.14)(Figure-8)indicating change in crystallinity respectively. The change in relative intensities of their XRPD peaks attributed to different crystal habits and arrangement of molecules indicating formation of new cocrystal.

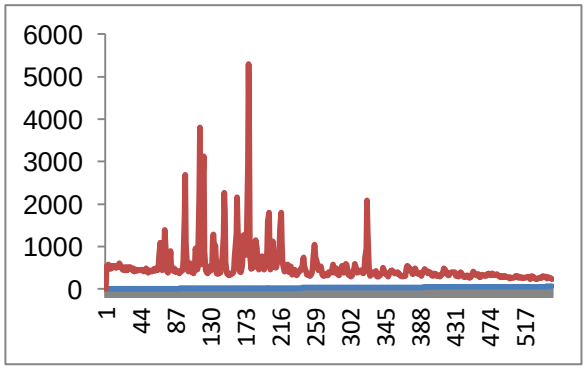


Figure -7: XRD fenofibrate

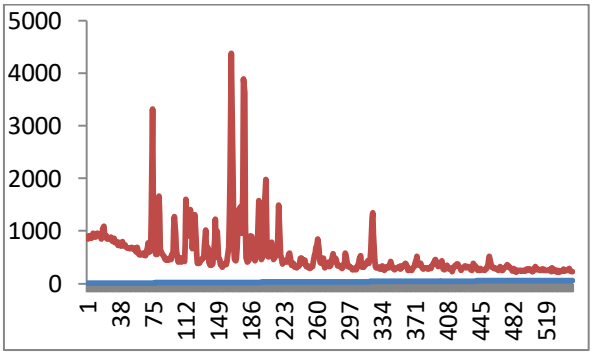


Figure -8: XRD fenofibrate-tartaric acid cocrystal

Drug content

Percentage drug content of fenofibrate-tartaric acid cocrystal was found to be 78.67%W/W.

***In-Vitro* Drug release:**

The *in vitro* dissolution profiles of the cocrystal were compared with that of pure fenofibrate (figure-9). The *in vitro* dissolution rate of cocrystal was increased compared to the drug. Pure drug shows 40.55% drug release after 60 min, whereas cocrystals show 95.8% . The high dissolution rate of prepared cocrystal can be attributed to change in crystallinity of fenofibrate due to possible hydrogen bond interaction with coformer. The antisolvent addition method produces non-hygroscopic solid form of fenofibrate cocrystal with markedly enhanced dissolution rate.

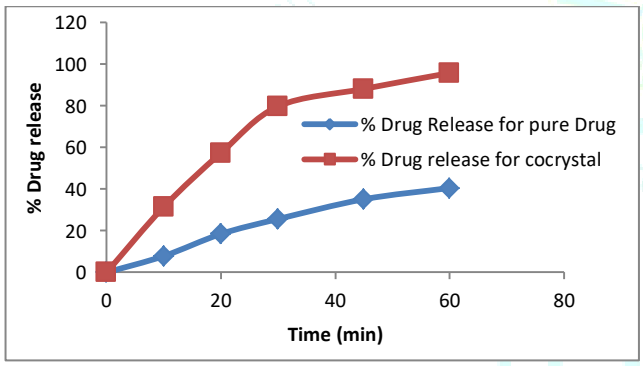


Figure 9: *In vitro* drug release from fenofibrate pure drug and fenofibrate-tartaric acid cocrystal.

***Ex-vivo* permeation study:**

Pure fenofibrate showed 15.98% absorption while Fenofibrate-tartaric acid cocrystal showed 70.1% absorption. Increase in the absorption might be due to the increase in solubility and dissolution rate. (Figure -10)

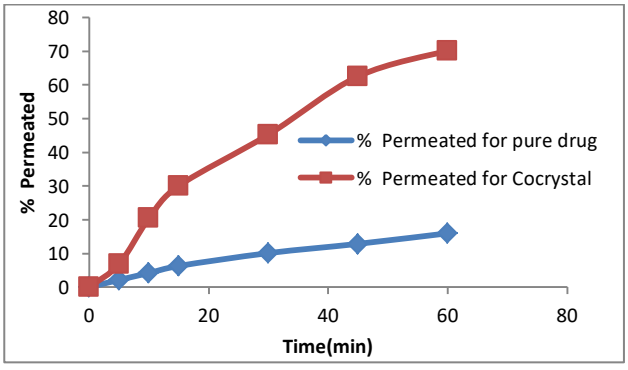


Figure 10: E*x-vivo* permeation studies using everted chicken intestine

CONCLUSION

The present study was aimed to investigate the use of Molecular docking in prediction of cocrystal formation between fenofibrate and coformers, and evaluate the prepared cocrystals by DCS, IR ,XRPD along with ***in-vitro*** drug release and ***ex-vivo*** permeation study of cocrystal. The investigated approach was effective in predicting coformer for fenofibrate in preparation of cocrystal.

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