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In Silico Screening of Coformers, Design and Characterization of Novel Etodolac Co-crystals

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ABSTRACT

Co-crystallization offers an alternative that has enormous potential to provide new stable structures with improved pharmacokinetic properties of an Active Pharmaceutical Ingredient (API). Pharmaceutical co-crystals are multicomponent solid forms made up of neutral molecules of API and pharmaceutically acceptable coformers in a crystal lattice. Co-crystals are increasingly popular in pharmaceutical industry since they extend an opportunity to improve and tailor the physicochemical properties of a given API without affecting the intrinsic activity of the molecule. This is particularly important in case of BCS ClassII drugs having poor aqueous solubility and bioavailability. Etodolac,a BCS class II drug is taken as model drug. Here a very effective insilico screening method is developed for the virtual screening of coformers. Eight coformers from the GRAS list were screened. Binding affinity and possibility of Hydrogen bond interaction were determined computationally.The coformer which showed good binding score was selected for further study. Co-crystal was prepared by solvent assisted grinding. It was characterized by FTIR, DSC TGA, Powder XRD and SEM.Equilibrium solubility studies were also conducted. The prepared cocrystals showed remarkable improvement in aqueous solubility. The insilico screening method developed is a very useful computational tool for improving the efficiency of coformer screen by limiting the candidate coformers to be studied experimentally to only those compounds which show good binding score.

INTRODUCTION

Physical property improvement is of particular interest to Active Pharmaceutical Ingredients (API) as majorityof the drugs are delivered as solid forms [1]. Nowadays most drugs are formulated as salts due to their aqueous solubility compared to their parent forms. But this salt formation is limited to APIs that possess an ionizable site for proton transfer. In such cases pharmaceutical co-crystals are of particular interest since they offer the advantage of generating diverse array of solid forms of API that lack ionizable functional groups needed for salt formation. Co-crystallization is a promising technique for drug development to modulate physicochemical properties of an API like poor aqueous solubility and bioavailability [2]. Co-crystal formation with suitable coformers has the potential for physical property manipulation like improvement in tabletability, reduction in hygroscopicity, stability, aqueous solubility etc. via modification of the underlying crystal structure. Pharmaceutical co- crystals are multi component systems composed of a neutral API and stoichiometric amount of a pharmaceutically acceptable coformer held together in a crystal lattice. In a co-crystal,components are held together via nonionic interactions like hydrogen bond, Pi - Pi interactions and vanderwaals forces. Among these hydrogen bond acts as key driving force to promote the molecular recognition which is required in the formation of co-crystals [3]. Effective formation of hydrogen bond between functional groups in API and complementary groups in coformer results in stable co-crystals. Unique advantage of co-crystals is that they not only retain the pharmacological activity of the API but also the API can be tailored to offer desired properties like superior solubility, stability and bioavailability. More than 40% of marketed drug and also about 80% of APIs under development belong to BCS Class II. BCS Class II drugs are of greater interest to researchers because its solid state, formulation, and physical characteristics are likely to have a significant influence on its

solubility and ultimately bioavailability. Etodolac, an NSAID belongs to BCS Class II is taken as model drug for the study. It has very low aqueous solubility and a high variability in bioavailability is seen. Literature survey shows preparation of a few number of co-crystals of etodolac in the last decade. In all these selections of coformers were done by trial and error which results in lot of wastage of raw materials and time. So here an attempt is made to select the coformer by screening through molecular docking using molecular modelling simulation software PyRx [4]. PyRx (Vina) is a bioinformatic tool which is used to perform in silico docking of proteins with a ligand [5]. Here it is used as a virtual screening tool to screen the coformers which are likely to bind with drug or API molecule to form co-crystals.[6].Using this software, probability of forming hydrogen bond between the API and coformer is predicted and the stability of the bonded structure is also given as docking score.Eight coformers were selected from GRAS (Generally Regarded As Safe) list and were virtually screened and the coformer succinic acid which showed good binding score was selected for further study. cocrystal was prepared by mechanosynthesis and was characterized.[7]. Its aqueous solubility was also evaluated and compared with that of parent drug.

MATERIALS AND METHOD

Personal Computer: HP Laptop, Intel core i5, Windows 10 Operating System

Programmers: Open Babel 2.3.2/ GUI, PyRx(Vina) Virtual Screening Tool and PYMOL

Etodolac 99.7% and Succinic Acid 99.8 % purity purchased from TCI Chemicals (India) Pvt. Ltd., Methanol AR Grade purchased from Merk.

Molecular Modelling and Docking Studies

Drug and Coformers Preparation

The 3D structure of etodolac and coformers were retrieved from Pub Chem (https://pubchem.ncbi.nlm.nih.gov/). The structures were saved in comfortable format(.sdf). Using Open Babel tool, the format was converted into pdb file format(Open Babel 2.3.2/GUI). The energy minimized structures were used for docking studies. The files were opened in PyRx(Vina) Virtual Screening Tool and they were converted into pdbqt format.

Molecular docking

The binding conformations between the selected coformers and the API were analyzed with the help of Auto DockVina docking algorithm [6]. All the calculations in docking were performed using Lamarckian Genetic Algorithm method. After docking, the pose with least binding energy was selected as the best confirmation. Docking was repeated for eight times. The interactions between the drug and the coformers were visualized using Pymol software. Parameters observed were number of hydrogen bonds and binding affinity (Ei (kcal/mol)).

Preparation and Characterization Studies

Co-crystals were prepared by solvent assisted grinding method[8].1:2 co-crystals were prepared using 0.359 gm etodolac and 0.294 gm succinic acid. They were ground together in an agate mortar for 20 minutes using 5 drops of methanol. Co-crystals were completely dried at room temperature. It was subjected to preliminary characterization studies and aqueous solubility determination.

Characterization

Fourier Transform Infrared Spectroscopy

FTIR spectra of Etodolac and co-crystalswere taken in the range 400-4000 cm^{-1} using Jasco-4200 type A spectrophotometer employing KBr Pellet method.

DSC TGA Analysis

Thermal analysis is an important tool used to study the properties of co-crystals as the temperature is changed. DSC TGA thermogram of etodolac, coformer and co-crystals were recorded in the range 30 to 800°Cin a differential scanning calorimeter. The heating was set at 10°Cper minute.

Powder XRD

Powder X Ray Diffractogram of drug, coformer and co-crystals were recorded at room temperature with Philips X pert Pro X- Ray diffractometer. Voltage 40 KV. The diffraction patterns were recorded from 10 ° to 70 ° at an angle 2Θ.

SEM

The morphological characters of co-crystalwas examined under Scanning Electron Microscope. The sample was loaded on aluminum stub with carbon adhesive tape. The sample was scanned at a voltage of 10 KV.

Solubility studies

Equilibrium solubility studies of etodolac and co-crystals were performed using shake flask method [9]. Absorbance of the drug and cocrystals were determined UV spectrophotometrically at 280nm [10]

RESULTS

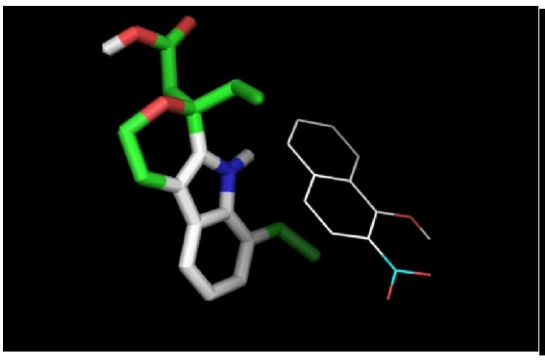
The coformers selected in the work were 1-Hydroxy-2-naphthoic acid, Benzoic acid, Caffeine, Isonicotinic acid, Nicotinamide, Succinic acid, Theophylline and Urea.

Binding affinity of the drug with coformers and number of possible hydrogenbonds are shown in table 1. Fig 1 shows docking of Etodolac with coformers.

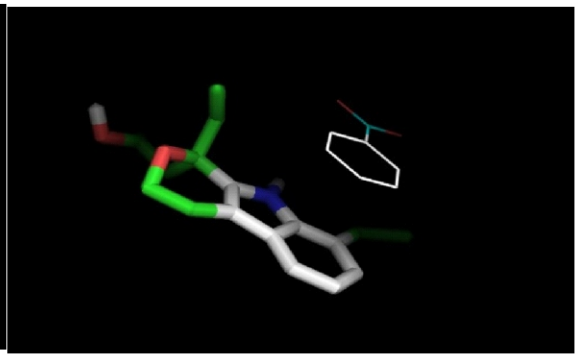
Out of the eight coformers screened for hydrogen bonding, succinic acid and isonicotinic acid were proved to be goodcoformers with least binding energy i.e. -2.3kcal/mol. Of this succinic acid was selected for the preparation of co-crystals and characterization studies.

FT IR spectrophotometry

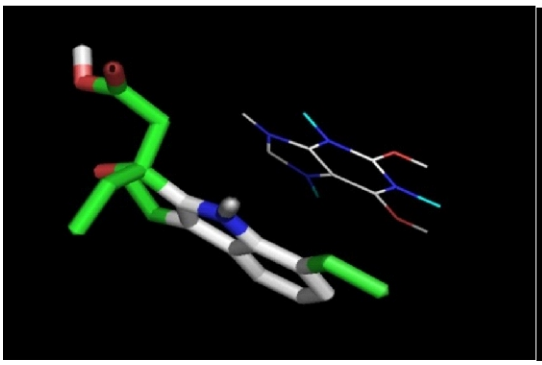
In fig 2 the FTIR spectrum of drug shows prominent peaks at 3345 (N -H stretching) , 2971 (O-H),1743 (C= O) .In fig 3 the FTIR spectrum of co-crystal shows prominent peaks at 3344(N -H stretching) ,2931(O-H group of carboxylic acid),1731 (C=O stretching)and 1693 (N-H bending). In fig 4 the stretching vibrations of -C=O shows a bathochromic shift from 1743cm-1 to 1731cm-1 and there is a broadening of the peak showing the involvement of carbonyl group in hydrogen bond formation. The -NH stretching frequency at 3345cm-1 is shifted to a lower frequency and the intensity is very much reduced. Absence of sharp peaks at 3345cm-1 and 2971cm-1 in the co-crystal spectrum (fig4) may be due to formation of C-O…H-N&OH...N-H hydrogen bonds [11]



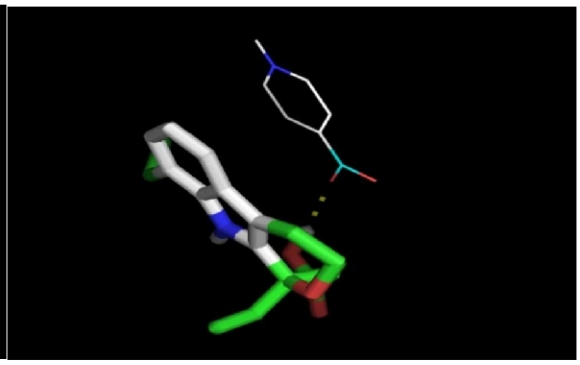
1-Hydroxy- 2-naphthoic acid and Etodolac



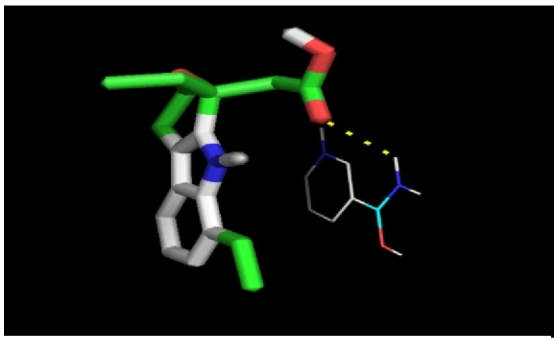
Benzoic acid and Etodolac



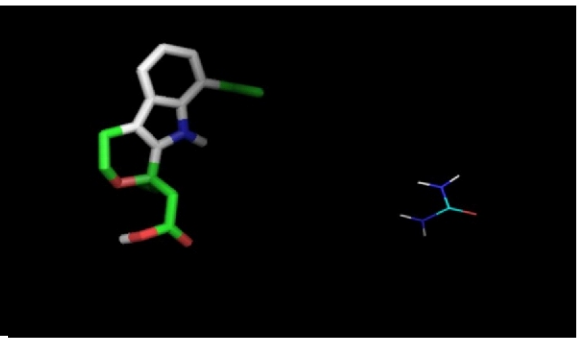
Caffeine and Etodolac



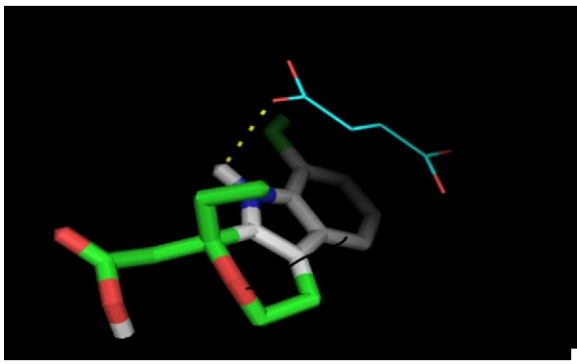
Isonicotinic acid and Etodolac



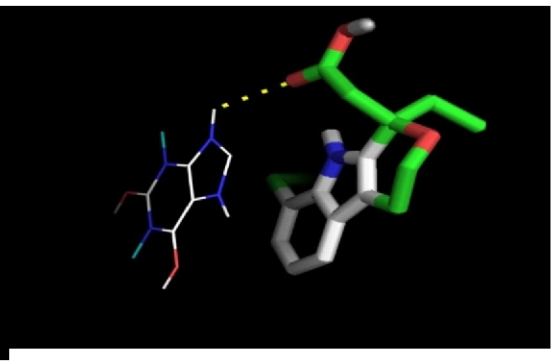
Nicotinamide and Etodolac



Urea and Etodolac



Succinic acid and Etodolac



Theophylline and Etodolac

Figure 1

Table 1 : Binding affinity and number of hydrogen bond interaction of different coformers with Etodolac

Coformers	Ligand	Binding Affinity (kcal/mol)	Number of hydrogen bond interactions
1- hydroxy-2-napthoic acid	Etodolac	-2.2	0
Benzoic acid	Etodolac	-2.1	0
Caffeine	Etodolac	-2.1	0
Isonicotinic acid	Etodolac	-2.3	1
Nicotinamide	Etodolac	-2.2	1
Succinic acid	Etodolac	-2.3	1
Theophylline	Etodolac	-1.1	1
Urea	Etodolac	-1.1	0

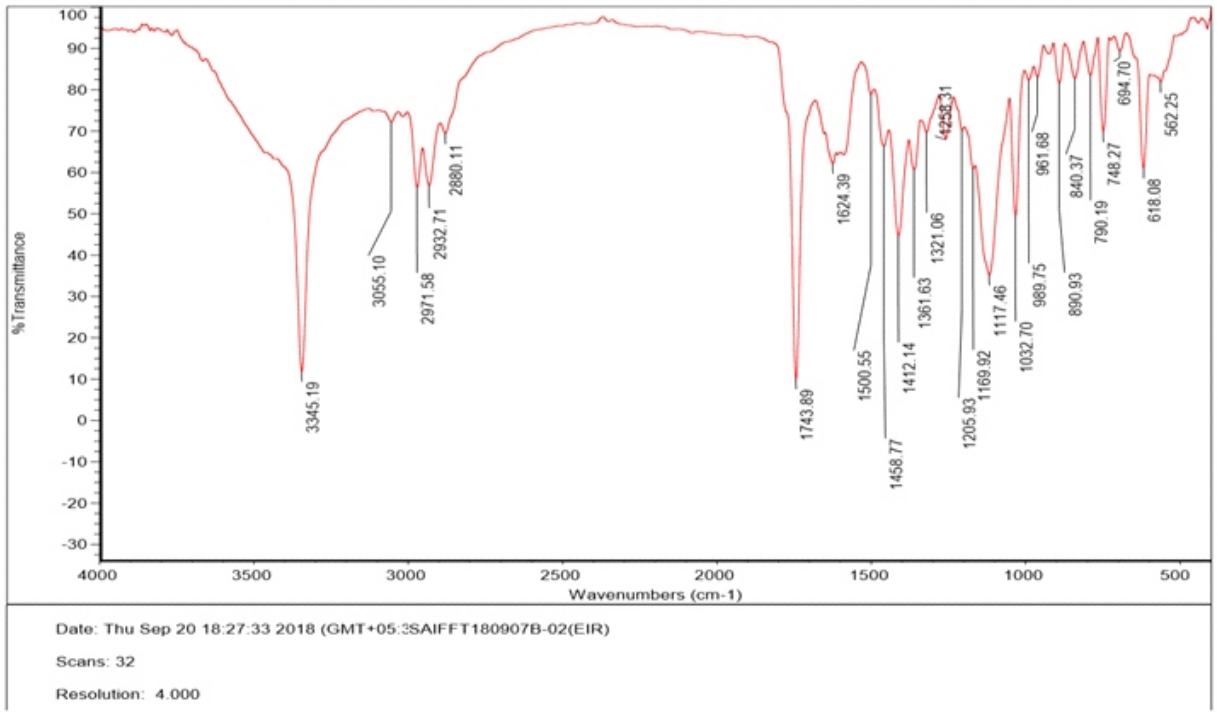


Fig. 2 : IR Spectrum of Etodolac

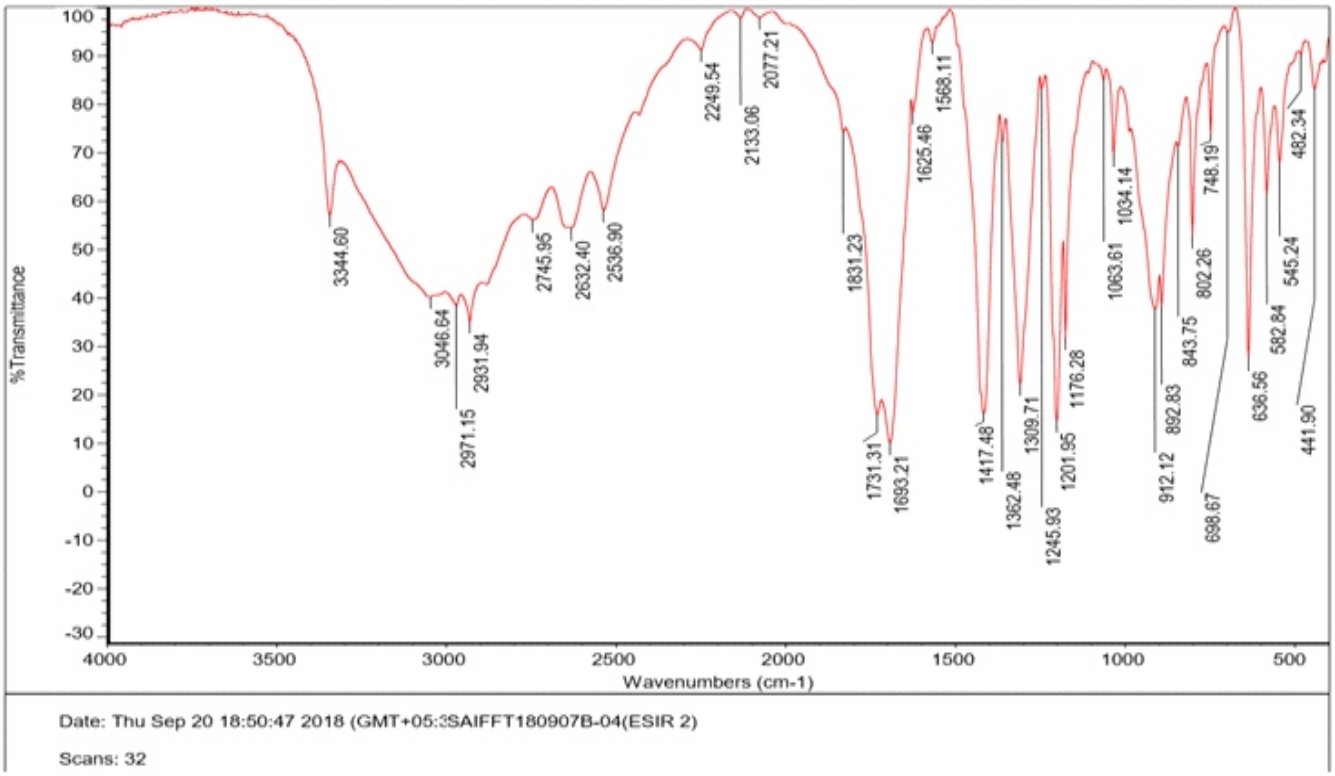
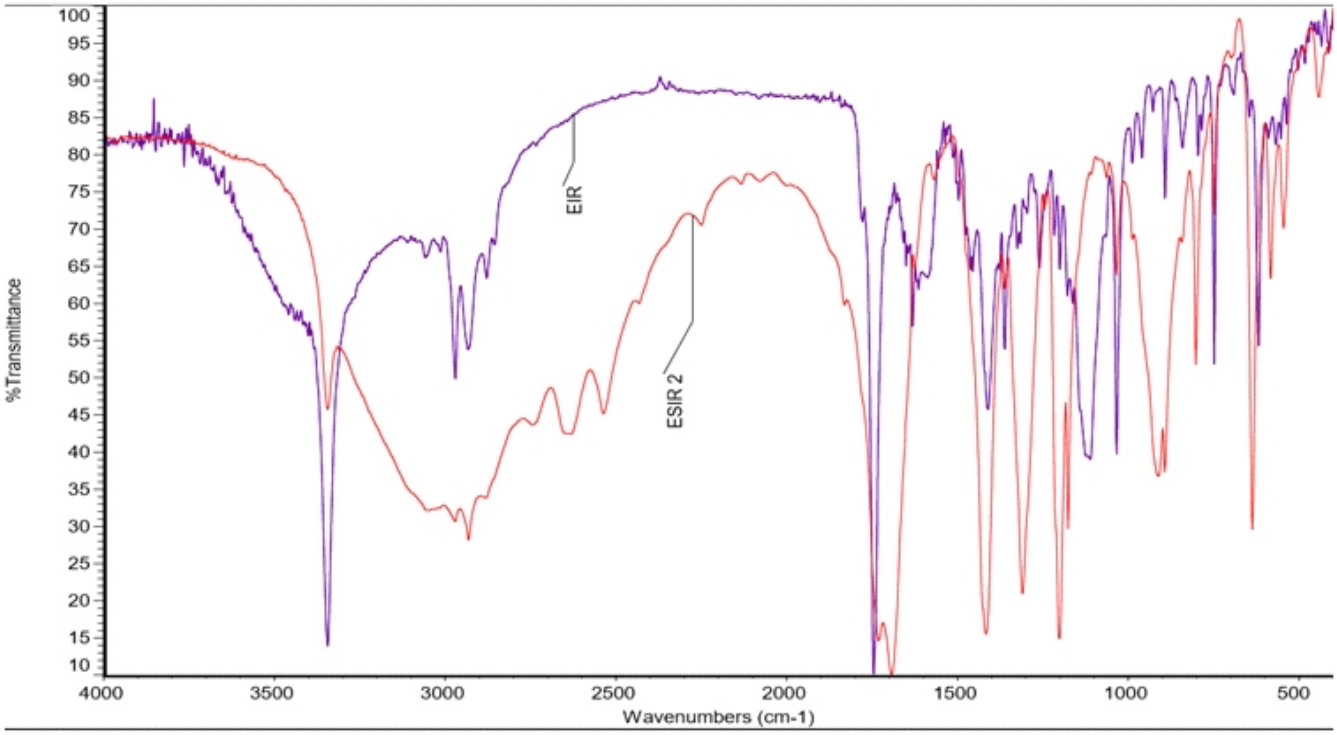


Fig. 3 : IR Spectrum of Etodolac Succinic acid co-crystals



EIR-Etodolac, ESIR2- Co- crystal

Fig. 4 : Overlay spectrum of the drug and co-crystal

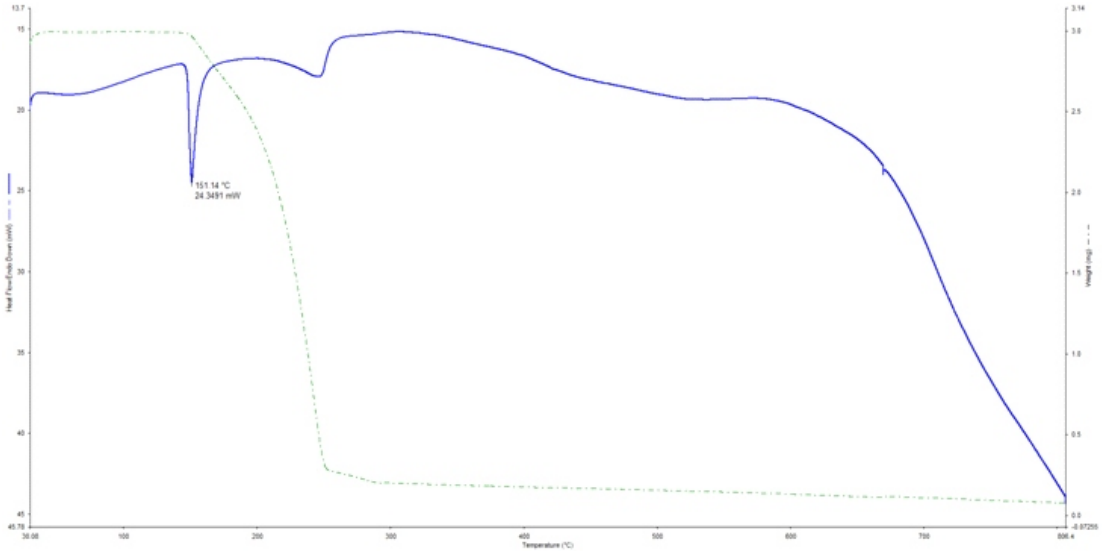


Fig. 5 : DSC TGA thermogram of Etodolac

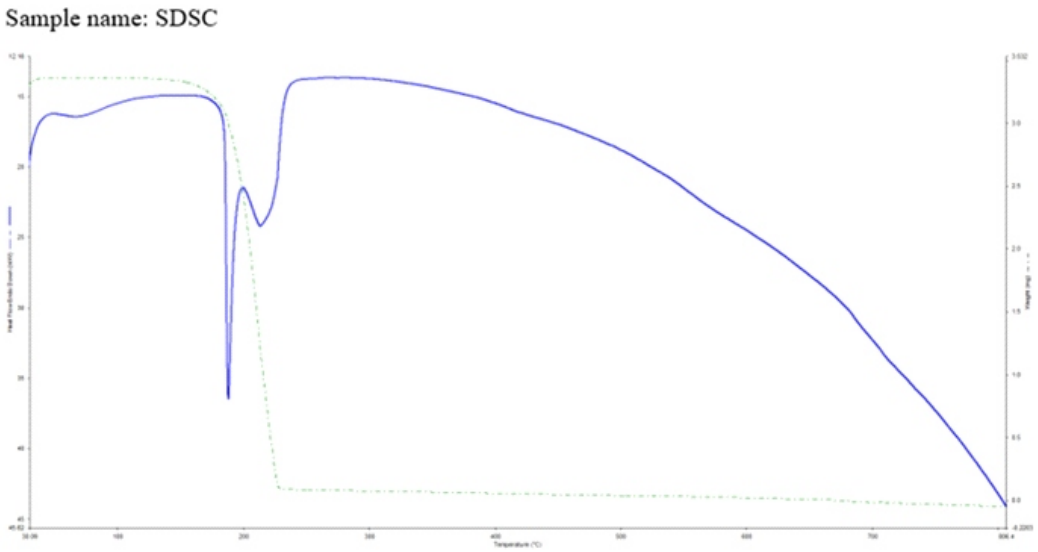


Fig. 6 : DSC TGA thermogram of Succinic acid

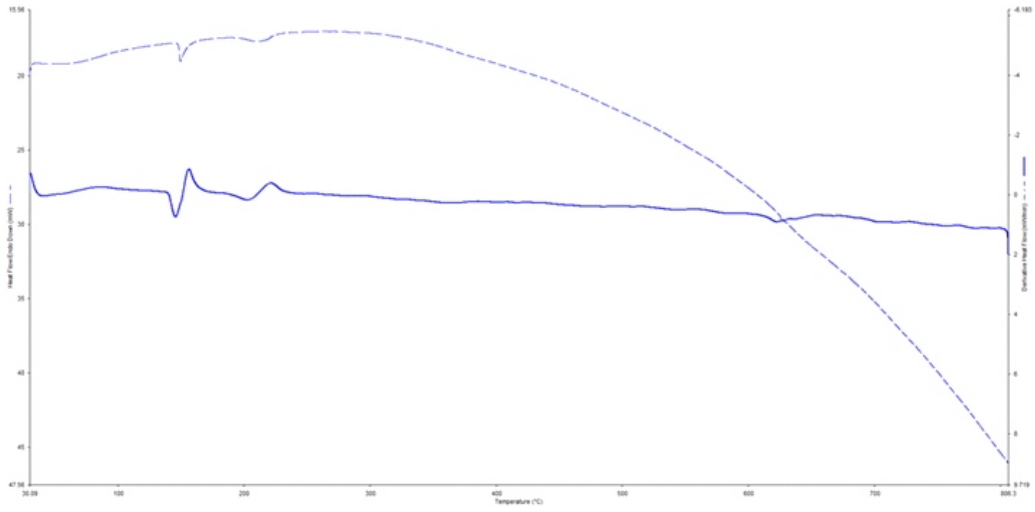


Fig. 7 : DSC TGA thermogram of the cocrystal

DSC TGA

The thermogram of etodolac fig 5 shows a sharp endothermic peak at 151.5°C which corresponds to its melting point. Thermogram of succinic acid fig 6 shows a sharp endothermic peak at 181°C corresponds to its melting point. But thermogram of cocrystal in fig.7 is broad and endothermic peak is observed at 160°C which shows a change in melting point and also it is evident that the cocrystal formed is thermodynamically stable.

Powder XRD

Powder XRD finger print matching exhibited new diffraction lines for cocrystal. Unique XRD pattern distinguishable from the drug and coformer was observed for the co-crystal.Diffractogram of Etodolac fig 8 shows characteristic peaks at 2θ values 10,14.61,19.66,21.756,30.68 and 38.96. The diffractogram of succinic acid fig 9 shows prominent peak at 20.88,25.19,32, and a less prominent peak at 41. But in the diffractogram of co-crystalfig 10a number of new peaks having high intensityis seen at 28,29,39,45,48,49,51 which shows the formation of a new crystalline phase [7].

Scanning Electron Microscopy

The co- crystals were found to be rectangular in shape with smooth surface.

Solubility Studies

Aqueous solubility of etodolac was found to be 0.015mg/ml

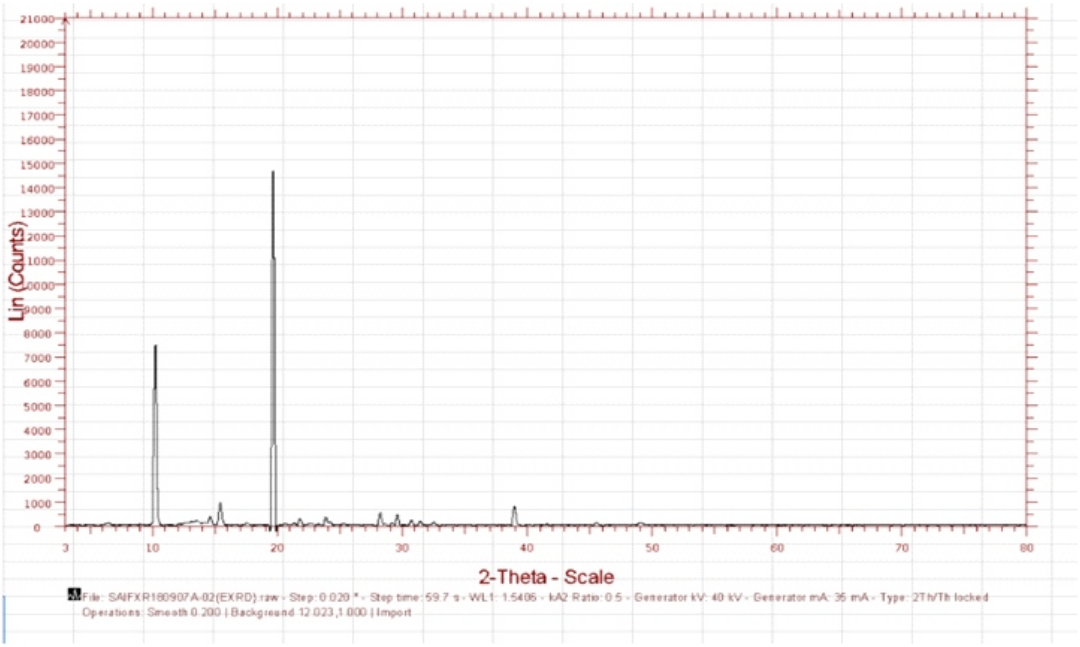


Fig. 8 : Powder XRD of Etodolac

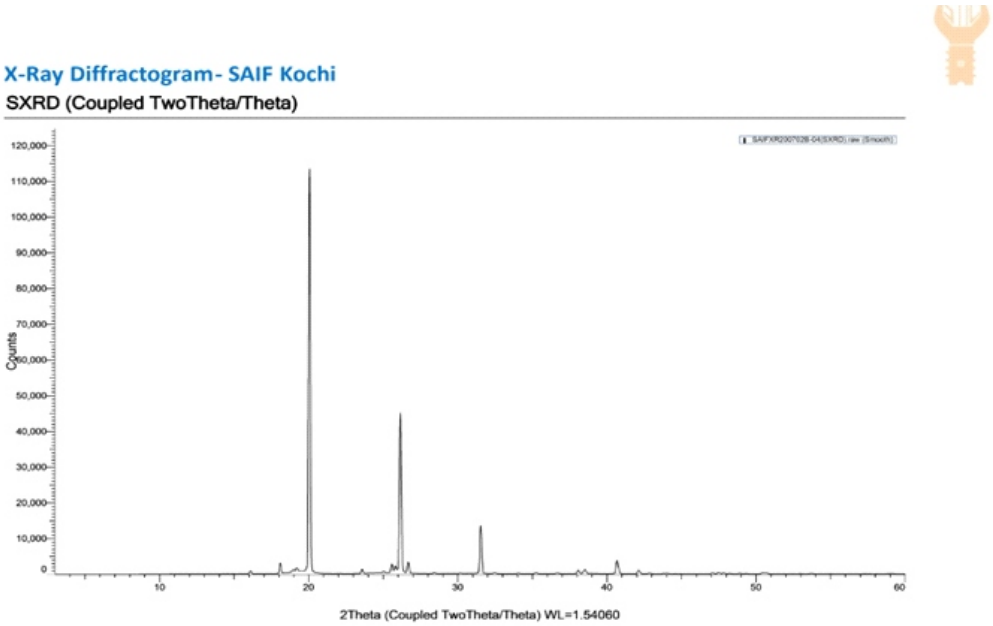


Fig. 9 : Powder XRD of succinic acid

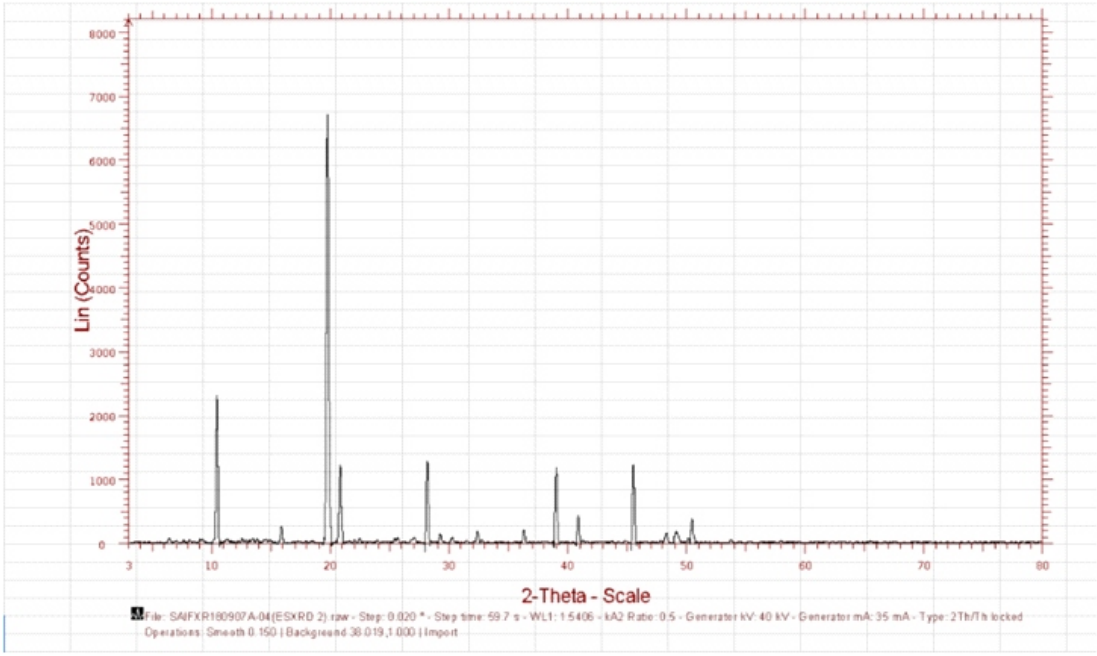


Fig. 10 : Powder XRD of Etodolac Succinic acid co-crystal

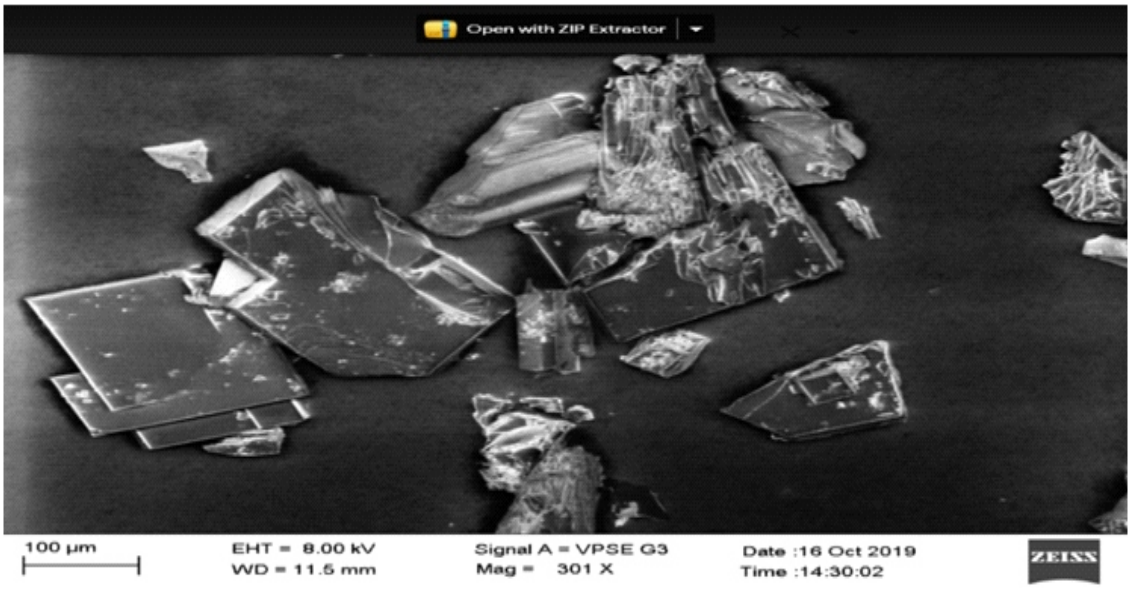


Fig. 11 : SEM images of the cocrystal

and that of co-crystal was determined to be 0.075mg/ml. This increase in solubility may be due to the hydrogen bond formed between etodolac and succinic acid in the co-crystal [7].

DISCUSSION

Based on in silico screening, the coformer succinic acid which showed good binding score was selected and the co-crystals were prepared by solvent drop grinding method, which is a sustainable green approach. The changes in the position and intensity of stretching and bending vibrations in the IR spectrum of cocrystal confirms the formation of new hydrogen bonds especially for -COOH and- NH. The pyrrole NH acts as hydrogen bond donor and two C= O groups in the coformer act as hydrogen bond acceptors which offer the formation of O--H---O,N-HO ,O-HN hydrogen bonds[11]. Single endothermic transmission of co-crystal is attributed to the melting temperature of pure co-crystal material which is entirely different from the starting components. In PXRD also distinct diffraction pattern is exhibited by the co - crystal. SEM studies reveal the smooth and crystalline morphology of produced co-crystals.Co-crystal solubility depends on the complexation behavior of the components and its interaction with the solvent. It is reported that highly soluble coformers produce cocrystals with higher solubility [11]. Here succinic acid has multifold solubility compared to API. The cocrystal shows higher solubility compared to API due to enhanced solute solvent interaction.

CONCLUSION

Insilico screening method of coformers by Auto Dock using

PyRx (Vina) is an effective tool to virtually screen a library of compounds. This is a very useful computational tool for improving the efficiency of coformer screen by limiting the candidate coformers to be studied experimentally to only those compounds which show good binding score. Succinic acid on insilico screening showed good binding score. Hence co- crystals of etodolac succinic acid were prepared and characterized by various analytical methods like FTIR, DSC TGA and Powder XRD, Surface morphology was studied by SEM. Equilibrium solubility studies were also conducted. Co-crystallization is a highly probable alternative to change the physical properties of etodolac and to improve the pharmacokinetic properties of its dosage forms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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