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Research Article

Fabrication and Solid State Characterization of Ticagrelor Co-Crystals with Improved Solubility and Dissolution

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ABSTRACT
In the present study a new co-crystal of Ticagrelor with L-Tartaric acid has been prepared with improved solubility. Ticagrelor is a class VI drug with poor solubility and permeability; hence an attempt has been made to improve its solubility by co-crystallization technology.A co-crystal is a structurally homogeneous crystalline material containing an API and the co-former in definite stoichiometric amounts. In this study the conformer selected was L-Tartaric acid based on ease of hydrogen bond formation. The co-crystal of Ticagrelor with L-Tartaric acid was prepared in different ratios (1:1, 2:1, 1:2). Ticagrelor formed stable co-crystals in the ratios 1:1&2:1. The formation of co-crystal was confirmed by FTIR, DSC and PXRD. The dynamic solubility of co-crystals in the ratios 1:1 and 2:1 was increased by approximately 2.7 and 2.6 fold respectively as compared to pure drug. The in-vitrodissolution study demonstrated a 1.5 fold increase in the solubility for selected TIC:L-TAR (1:1) as compared to its TIC active pharmaceutical ingredient and TIC physical mixture.

Keywords: Ticagrelor, L-Tartaric acid, slow evaporation method, Dynamic solubility, co-crystal.

INTRODUCTION

The effectiveness of a drug depends on the bioavailability which ultimately depends on the solubility of the drug. Solubility and a suitable bioavailability is important for desired pharmacologic response to be shown. Though there are so many techniques to improve the solubility, co-crystallization is one of the new approaches. These co-crystals include molecular complexes, channel compounds, clathrates[1]. Co-crystals are structurally crystalline, neutral molecules which contain two or more components present in definite stoichiometric amounts. Pharmaceutical co-crystals contain an active pharmaceutical ingredient (API) and a co former which may/may not have pharmacological activity[1]. Ticargrelor (figure 1) is an oral antiplatelet agent acting directly and reversibly at p_2y_{12} receptor. It belongs to cyclo-pentyl-triazolo-pyrimidines family & chemically it is [(1S,2S,3R,5S)-{(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}-(propylthio)-3H[1,2,3]-triazolo[4,5-d]pyrimidine-3yl]-5-(2-hyroxyethoxy)cyclopentane-1,2-dio][3]. The Ticargrelor drug belongs to BCS class IV molecule, poorly soluble and permeable[2]. The absolute bioavailability of Ticargrelor is in range 30-42%.The half life of drug is 7.5-8 hours. Enhancement of the solubility of Ticargrelor by formulating solid dispersions with hydrophilic carriers based solvent evaporation technique was carried out by K. Ramesh, B. Chandra Shekar, and P. Khadgapathi[2]. There are also two patents on Ticagrelor co-crystals with aspirin and nicotinamide. This current research work is aimed at enhancing the aqueous solubility and thereby the rate of in-vitro drug release profiles of Ticargrelor by co-crystal technology.

MATERIALS AND METHODS

Materials

Ticagrelor was provided as a gift sample by Apotex pharmachem India**.** Acetonitrile and methanol (HPLC grade) was provided by Finar chemicals limited (Ahemdabad, India). L- Tartaric acid was purchased from S.D. Fine Chem. LTD, Boisar. HPLC grade water was produced in the laboratory by a Milli- Q purification system (Siemens AG, Schuhstr. 60, and 91052 Erlangen, Germany).

Methods

Preparation of co-crystals of Ticagrelor and L-tartaric acid

The binary co-crystals were prepared by various techniques like slow evaporation, dry grinding, slurry conversion and slow evaporation technique was selected to prepare the final batches of Ticagrelor co-crystals. A mixture of Ticagrelor and L-Tartaric acid were weighed in

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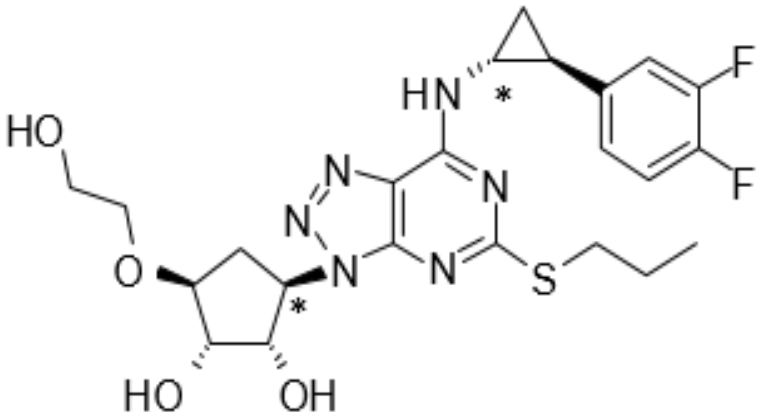

In the structural formula above, all chiral centres are in *S*-configuration except those marked * which are in *R*-configuration.

Figure 1: structure of Ticagrelor.

Table 1: Batches of co-crystal prepared by slow solvent evaporation technique.

S No	Batch	Coformer	Solvent	Technique	Ratio
1.	TIC:L-TAR01	L-Tartaric acid	Methanol	Slow solvent evaporation	1:1
2.	TIC:L-TAR02	L-Tartaric acid	Methanol	Slow solvent evaporation	2:1
3.	TIC:L-TAR03	L-Tartaric acid	Methanol	Slow solvent evaporation	1:2

Table 2: Melting endotherms Ticagrelor, Tartatic Acid and TIC:L-TAR01 cocrystal.

Sample	Melting endotherm
TIC_PURE	142.50°C
TAR_PURE	222.46°C
TIC:L-TAR01	90.87°C& 131.99°C

Table 3: XRD results of pure drug and co-crystals of TIC: L-TAR01.

Sample Id	2θ(degree)	d(angle)	Intensity
TIC_PUR	16.09	5.49	1856.72
TIC:L-TAR01	24.43	2.38	1043.1

Table 4: Saturation solubility data of the selected TIC:L-TAR01 co-crystal with pure TIC and physical mixture of TIC.

SAMPLE ID	Concentration in µg/mL
	24hours
TIC(PUR)	2.5
TIC(PM)	2.4
TIC:L-TAR (1:1)	6.8

Table 5: Invitro dissolution results of pure TIC, physical mixture and TIC: L-TAR01 co-crystal.

TIME	% DRUG RELEASE		
(min)	Cocrystal(1:1)	Pure drug	Phy-mix
0	0	0	0
10	1.81	BDL	BDL
20	14.54	13.98	7.6
30	18.60	20.91	14.83
45	25.57	23.76	17.00
60	32.69	24.98	20.65
75	37.27	25.63	25.07

different molar ratios of 1:1 (250mg of TIC and 73.8mg of
TAR), 1:2 (250 mg of TIC and 147.6mg of TAR) and 2:1(500mg of TIC and 73.8mg of TAR). Saturated solution of Ticagrelor and L-Tartaric acid were prepared separately in Methanol. The two solutions were then mixed and sonicated for 5 minutes. The solution was then poured into a Petri-dish and the solvent was allowed to evaporate at room temperature[5].

Solid state characterization studies

FTIR

Shimadzu FTIR – 8300 system (Kyoto, Japan) was used to obtain the spectra of the prepared samples. The spectrum was collected over a range of 4000 – 500cm^{-1}. (25 scans, resolution 4 cm^{-1}). Preparation of the disc involved dispersing the sample in KBr and then grindingwith applied pressure(1000psig). Unwanted bands were discarded from the respective spectrum.

DSC

Shimadzu TA – 60WS thermal analyzer was used to obtain DSC measurements. Required amount of sample(about 2mg) were placed in flat bottomed aluminium pans(0.1mm thickness) and then crimped with an aluminium lid. Then the samples were placed into sample holder and allowed to heat from 25 to 300 ˚C at a fixed heating rate(10 ˚C per minute) under a nitrogen flow (10cc/minute). The temperature calibration of the instrument was carried out using indium standard, the same heating rate and pan type used for the study. Heat flow and heat capacity signals were calibrated using powdered alumina(5mg, 100mesh) as a reference.

XRD

Rigakuminiflex 600 X-ray diffractometer (Rigaku Co., Tokyo, Japan), operated at 600 watts (X-ray tube), with a fixed tube current(15 mA) and a fixed voltage(40 kV) was used to achieve the X-ray powder diffraction pattern. The

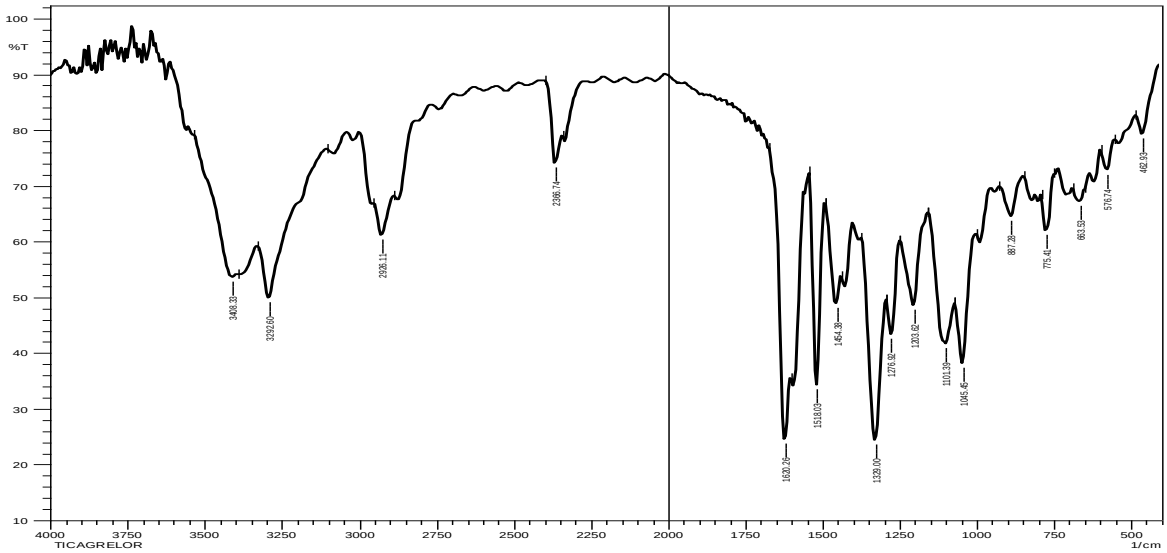

Figure 2a: FTIR spectra of pure Ticagrelor.

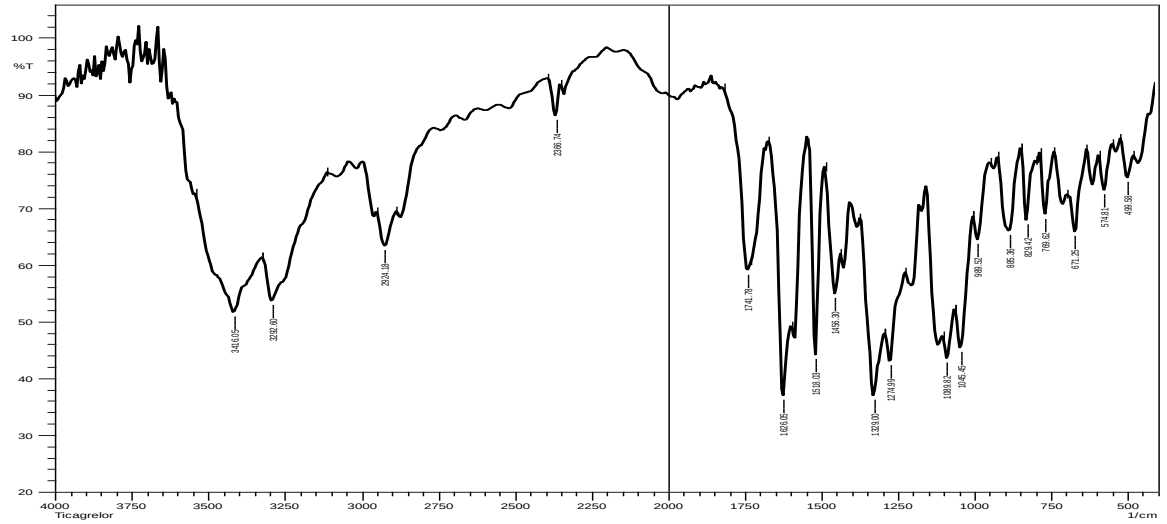

Figure 2b: FTIR spectra of Ticagrelor and DL-tartaric acid co-crystal (1:1)

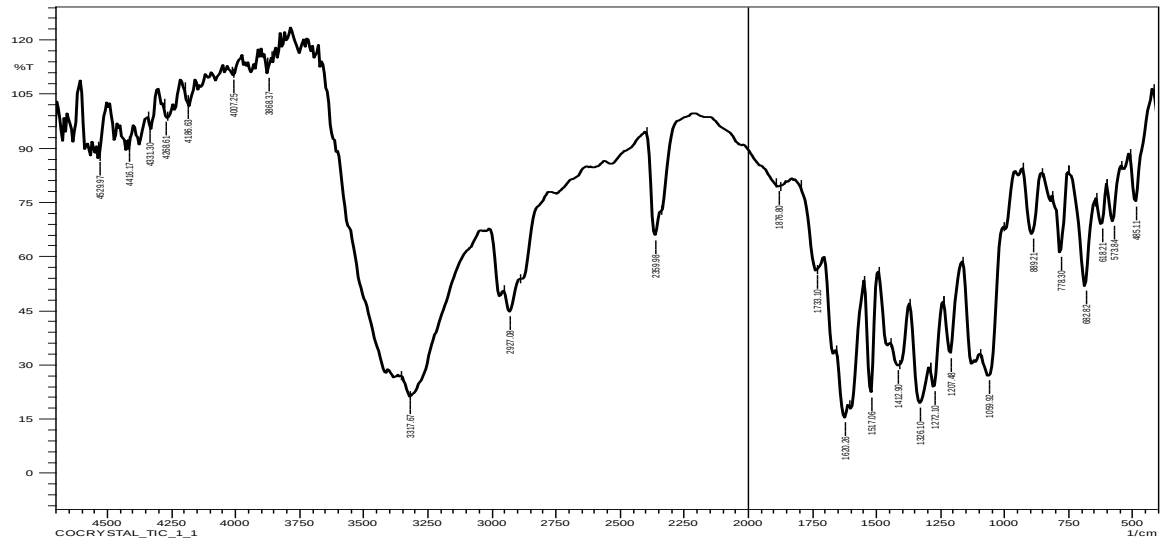

Figure 2c: FTIR spectra of Ticagrelor and L-tartaric acid co-crystal (TIC:L-TAR01).

X-ray beam(diffracted) was monochromated by a graphite monochromator and detection was carried out by a standard scintillation counter. Diffraction intensities were measured over a range of 5–80° (2θ).

Saturation solubility studies

Shake flask method was used to determine the equilibrium dynamic solubility of pure, physical mixture and co-crystals of Ticagrelor. USP phosphate buffer (pH 3) was used since it is reported in literature that Ticagrelor has highest solubility in phosphate buffer pH 3. Samples were prepared by adding excess quantities of each into injection vials containing 5mL of USP phosphate buffer (pH 3). Thereafter all the samples were shaken in Labtop orbital shaking incubator maintaining the temperature 37 ˚C at 150rpm. Samples were collected after 24 hours and centrifuged at 10,000 rpm maintaining the temperature 37 ˚C. The clear supernatant solution was separated and sufficiently diluted to fall in calibration range before injecting in the HPLC system. The samples were analyzed using reported HPLC method. The experiment was conducted in triplicate (n=3). The samples were quantified using reported HPLC method using UV detector at 254nm.

Dissolution

The pure, physical mixture and the optimized cocrystal(1:1) of Ticagrelor were accurately weighed sufficient to maintain the sink condition and were filled in hard gelatine capsules(size 2). Electrolab- Tablet Dissolution tester using basket apparatus at a rotational speed of 75 rpm and USP phosphate buffer (500 mL, pH = 3.0, 37 ˚C) + 0.2% Tween80 as a dissolution media was used to carry out dissolution. At intervals of 10, 20, 30, 45, 60 and 75, 5mL of samples were taken out and were replenished with same amount of dissolution medium. Test samples were then filtered through a membrane filter(4.5µm) and sufficiently diluted. Diluted sample was analysed by reported HPLC method mentioned in section

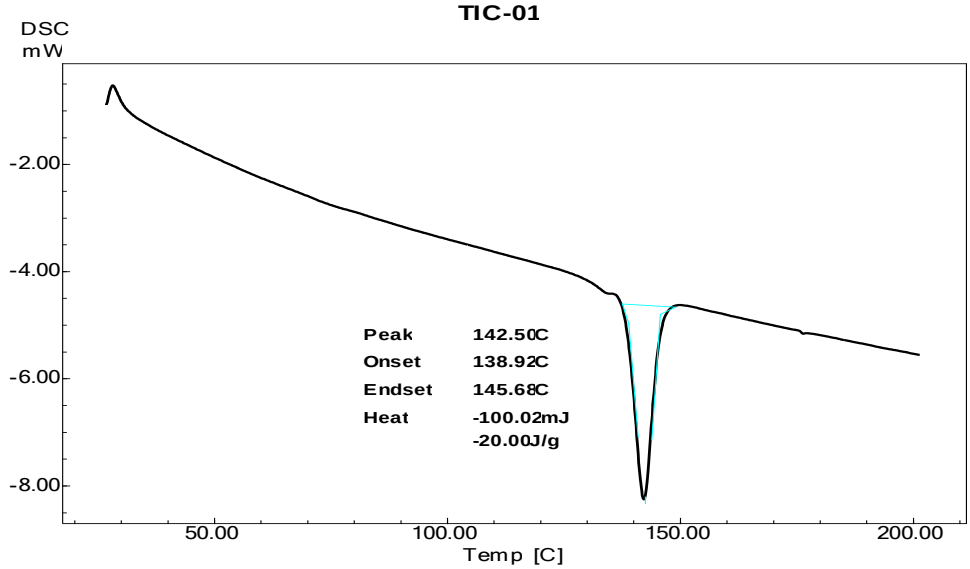

Figure 3a: DSC thermogram of pure Ticagrelor.

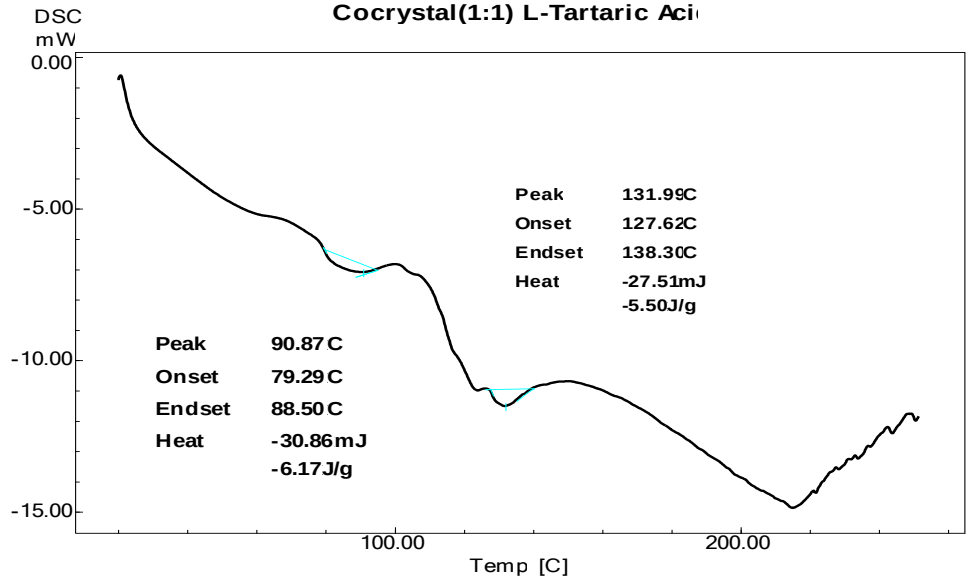

Figure 3b: DSC thermogram of TIC:L-TAR01.

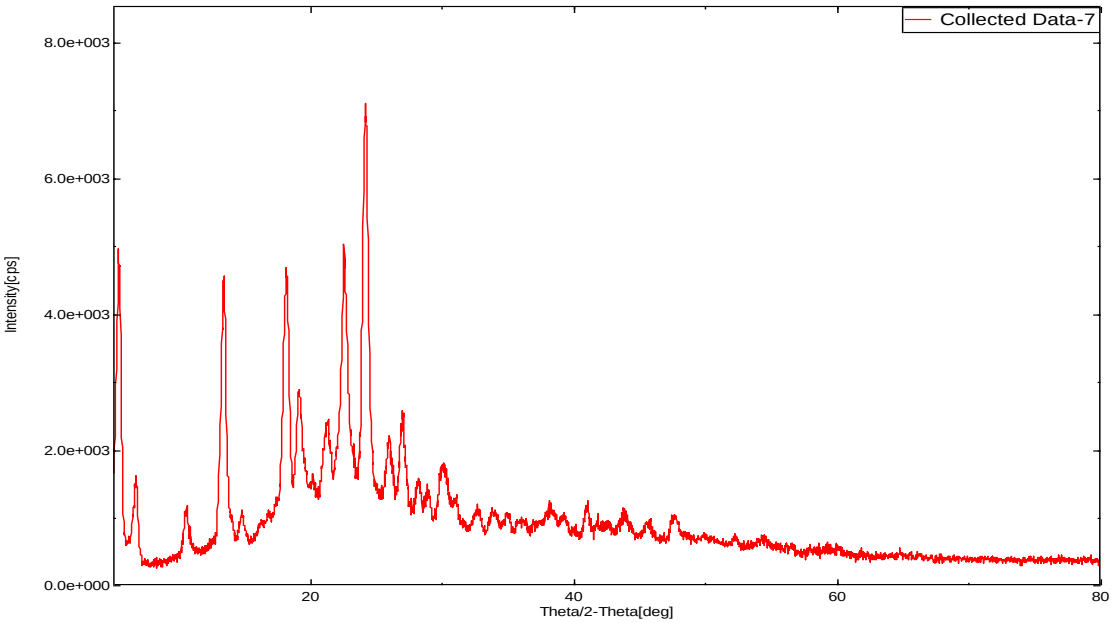

Figure 4a: The diffraction patterns of pure drug.

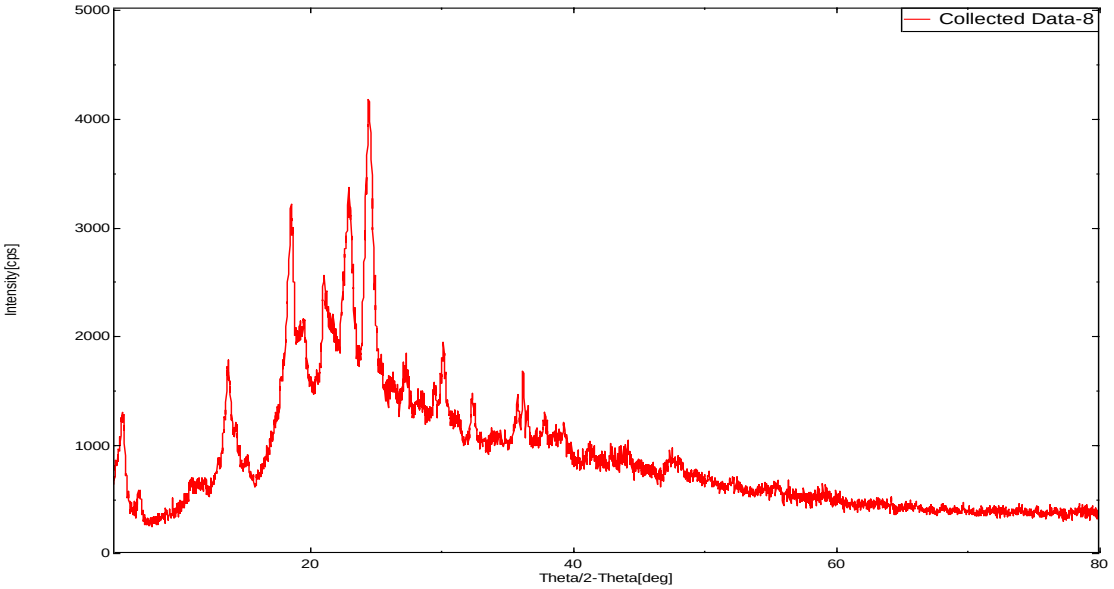

Figure 4b: The diffraction patterns of Ticagrelor and L-Tartaric acid co-crystals.

2.2.6 to determine the concentration.

Stability Studies

Chemical Stability

Accurately weighed quantity(10mg) of cocrystal and pure drug was taken in separate 10ml volumetric flasks and volume was made up to 10ml with acetonitrile(ACN). From the above solutions 10µl was taken into separate 10ml volumetric flask and the volume was made up with the same solvent. The resulting solution was analysed by reported HPLC method mentioned in section 2.2.6 to determine the concentration. The % assay was determined by dividing cocrystal peak area by the standard (pure drug) and multiplied by hundred. Also peak purity testing was done to make sure that no impurities are generated during the fabrication process.

Physical Stability

To determine the physical stability, the cocrystals were stored in a dessicator (with $CaCO_3$ at the bottom) at 25°C for 30 days and then analysed by XRD.

Chromatography

Cocrystals were analysed by Shimadzu HPLC (Kyoto, Japan), system controlled by LC solution software and equipped with a LC-10 ADVP(quaternary pump), SIL-10 ADVP (Auto injector), a SPD M-10A VP (photo diode array detector) and SPD-10Avp (UV detector). The separation was carried out on a C18 Vydac Monomeric 120A (5.0 micron, 250 x 4.6mm) column. 60:40 (v/v) Acetonitrile: water (pH 3.5) in the ratio was used as mobile phase(MP). 1000 ml of water (milli-Q), to this triethylamine(1ml) was added and pH was made to 3.5 ± 0.02 with 10 % Orthophosphoric acid. Filter water through a filter(0.45µ) using a filtration assembly(vacuum) and then degassed by ultrasonication for ten minutes prior to

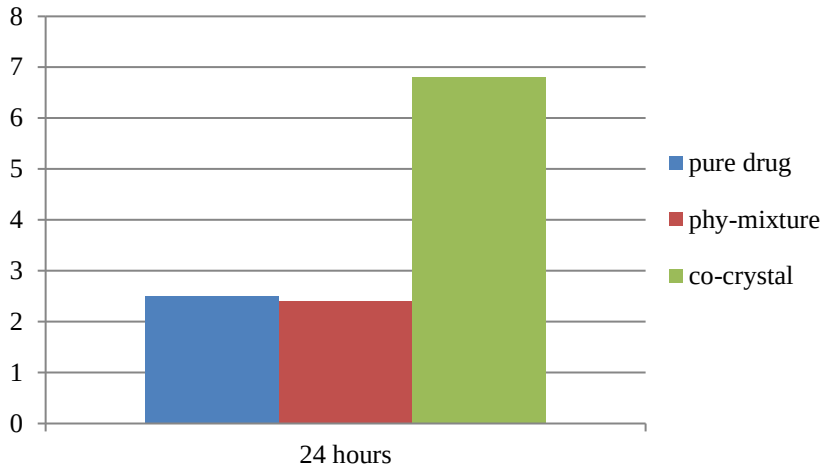

Figure 5: Saturation solubility data of the selected TIC:L-TAR01 co-crystal, pure TIC and physical mixture of TIC.

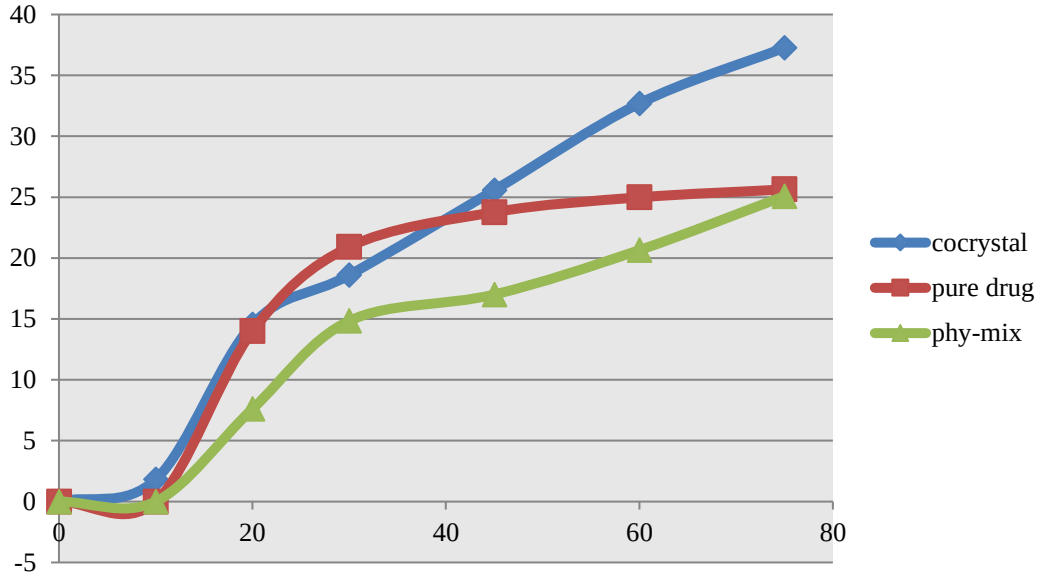

Figure 6: Dissolution studies of selected TIC: L-TAR01 co-crystal with pure TIC and physical mixture of TIC.

use. A flow rate of 1.0 ml/min was maintained and the column was maintained at 30ºC. PDA detector was used to estimate Ticagrelor. Sample was injected(20µl) and allowed to run time for 8 minutes.

RESULTS AND DISCUSSIONS

Co-crystals of Ticagrelor with L-tartaric acid were prepared by slow evaporation technique in ratios 1:1, 2:1 and 1:2. The co-crystal were the characterized by DSC, FTIR and PXRD which proved the formation of co-crystals. Saturation solubility was carried out by shake flask method and samples were analysed after 24 hours. Results showed a 2.7 fold increase in solubility (1:1) as compared to the pure drug.

Solid State Characterization

FTIR

Initially co-crystals of Ticagrelor were prepared with DL-Tartaric acid. FTIR spectra(fig-2b) revealed that co-crystals were not formed as the spectra was similar to that of pure Ticagrelor(fig-2a) with additional C=O stretching vibration (1737cm-1) of tartaric acid. The aromatic OH and secondary amine peak in Ticagrelor are of *R*-configuration (refer fig-1). Since L-configuration of tartaric acid corresponds to *R*-configuration of Ticagrelor, L-Tartaric acid was used as the co-former. On forming co-crystals with L-Tartaric acid the C=O stretching vibration (1741cm-1) in tartaric acid disappeared in the co-crystal and the peak broadening of secondary amine indicates hydrogen bond formation as C=O is an acceptor and NH are donors (fig-2c). Also no other interactions are possible due bulky(CH_3) and repelling (F) groups present in Ticagrelor which proves that interaction is only through the OH and NH groups of Ticagrelor.

DSC

The difference in the melting point of co-crystals when compared to pure Ticagrelor (142.50 °C) and disappearance of the first ordered transformation(melting endotherm) indicates interaction between the drug and the co-former. (Refer Table 2 &Figure. 3)

XRD

The interaction between the drug and the coformer is indicated by the difference in 2θ values of pure drug and

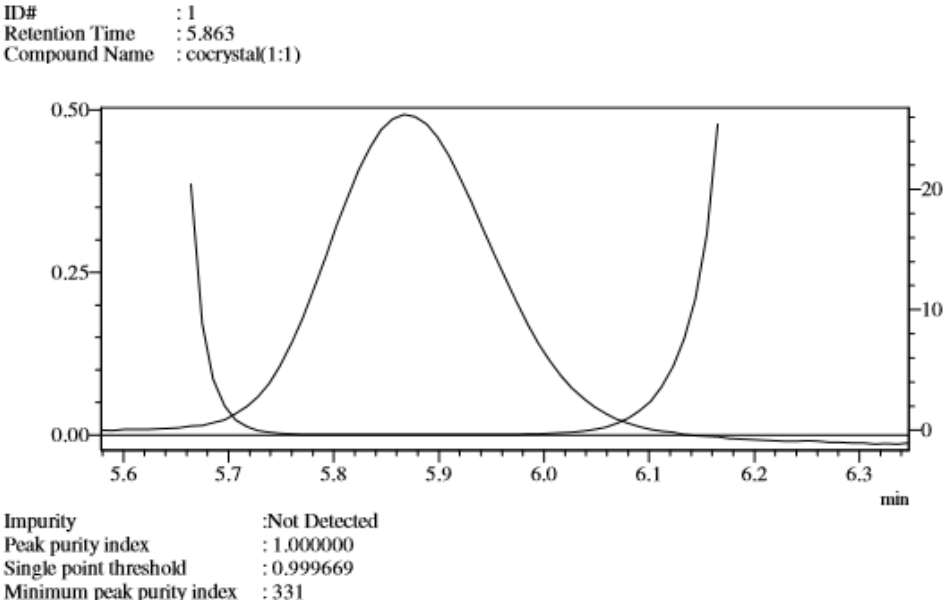

Figure 7: peak purity of TIC:L-TAR01Cocrystals.

cocrystals. A change in the differaction patterns were seen. The pure drug showed sharp peaks whereas the cocrystals did not show sharp peaks which suggests that there is interaction. Interarrangement of molecules is indicated by different peak locations of the cocrystals with respect to pure drug, hence proves formation of new crystalline phase. (Refer Figure-4, and Table 3)

Saturation solubility

The saturation solubility of pure Ticagrelor in USP buffer(pH 3.0, 37 °C) was found to be 2.5µg/mL after 24hours. Whereas the co-crystal form of Ticagrelor with L-tartaric acid in the ratio 1:1 prepared by slow evaporation showed dynamic solubility of 6.8µg/mL after 24 hours.Ticagrelor co-crystal (1:1) form shows approximately 2.72 fold increase in solubility as compared to its pure form andphysical mixture (TIC and L-TAR). The experiments were conducted in triplicates (n=3). (Refer Figure. 5 and Table.4)

Dissolution

The *in-vitro* dissolution study demonstrated a 1.5 fold increase in the solubility for selected TIC:L-TAR (1:1) as compared to its TIC active pharmaceutical ingredient and TIC physical mixture. Pure Ticagrelor showed a release profile with a maximum of 25.63% at 75 min. The % drug release at the end of 180 min was 37.27% for the co-crystal formulation(1:1).the physical mixture showed 25.07% drug release at the end of 75 min. The in vitro dissolution studies revealed a notable increase in the % drug release for co-crystals when compared with the Ticagrelor alone(figure-6, table-5).

Chemical Stability

The amount of drug present in Cocrystals was 105.12 ±0.14%. No impurities or degradation products were present in the Cocrystals as a peak purity index of 1.000000 was obtained, which shows that the drug is stable(refer figure7). Ticagrelor has a retention time of 5.8min.

Physical Stability

XRD results confirmed the stability of Cocrystals when kept in a dessicator (with $CaCO_3$ at the bottom) at 25°C for 30 days.

CONCLUSION

Based on the saturation solubility results, it was clearly obvious that the solubility of Ticagrlor cocrystals with L-Tartaric acid was on higher side as compared to that of pure Ticagrelor. Finally it could be concluded that the co-crystals of Ticagrelor by slow evaporation technique improved the solubility and thereby enhanced the rate of in vitro drug release. Therefore co-crystal formation by slow evaporation technique might be a useful strategy for increasing the therapeutic potential of Ticagrelor.

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