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Research Article

COCRYSTALS OF EFAVIRENZ WITH SELECTED COFORMERS: PREPARATION AND CHARACTERIZATION

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ABSTRACT

Pharmaceutical cocrystallization is a novel technique to alter the physicochemical properties of API to fine-tune with its bioavailability and solid state properties. This work deals with method preparation of cocrystals of efavirenz to enhance its aqueous solubility and characterizing the cocrystals employing various spectroscopic, X-ray and thermal method of analysis. Cocrystals of efavirenz were prepared with selected coformers of GRAS status using solvent drop grinding and solvothermal method. Equilibrium solubility profile of EFA-OXA and EFA-CITR shows an enhancement of 1.8 and 2.7 folds of solubility of efavirenz as compared to commercial sample. Sharp melting endotherm in DSC thermogram which was preceded by a broad desolvation endotherm due to unbinding of water molecule is confirmed by the mass loss in TGA thermogram. Increased numerical value of enthalpy of solution in the dissolution of cocrystals was observed as compared to pure drug and their physical mixture indicating involvement of hydrogen bonding. Thus desired solid forms as cocrystals were obtained without covalent modification and using eco-friendly methods of preparations.

Keywords: Cocrystals, Dissolution, DSC, Efavirenz, Enthalpy, Solubility, TGA and XRPD.

INTRODUCTION

Recent years have witnessed a rapid growth of interest in the design and synthesis of multi-component crystals in the context of pharmaceutical cocrystals (1).Cocrystals can be defined as crystalline complexes of two or more neutral molecular constituents bound together in the crystal lattice through noncovalent interactions, primarily hydrogen bonding (2).Pharmaceutical cocrystallization is a reliable method to modify physical and technical properties of drugs such as solubility, dissolution rate, stability hygroscopisity, and compressibility without altering their pharmacological behavior (1,3).The formation of a pharmaceutical cocrystal involves incorporation of an API with a pharmaceutically acceptable molecule in the crystal lattice. The resulting multi-component crystalline phase maintains the intrinsic activity of the parent API (4).

Cocrystal former employed in cocrystallization may be an excipient or another drug (5).A number of pharmaceutical cocrystals have been reported to date with cocrystal formers selected from the list of GRAS (generally recognized as safe) compounds which includes various food additives, preservatives and pharmaceutical excipients (6).

Pharmaceutical cocrystals provide an alternative to chemical modification of the drug substance as well as established salt, amorphous, solvate, polymorphic drug forms and inclusion complexes,all of which have limitations in their utility (7,8). Cocrystals can be considered for nonionizable drugs for which salts cannot be attained. Also, for ionizable drugs, the number of suitable cocrystal ligands can exceed the number of suitable counterions (9).

Efavirenz is a poorly water soluble BCS class-II drug (10),with low aqueous solubility of 9.2 µg/ml (11).Efavirenz inhibits the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Efavirenz acts allosterically by binding to a distinct site away from the active site known as the NNRTI (Non nucleocidal reverse transcriptase inhibitor) pocket (12).Pharmaceutical cocrystals of efavirenz were prepared with oxalic acid dihydrate and citric acid monohydrate (GRAS status) with the aim to improve its physicochemical properties (solubility and dissolution rate). Both oxalic acid dihydrate and citric acid monohydrate have high water solubility (14.3g/100 ml and 64.7g/100 ml respectively).Both these coformers contain hydrogen bond donor and acceptor groups, which can be used for designing cocrystals of efavirenz leading to improvement in solubility.

MATERIALS AND METHODS

Materials

Efavirenz (USP) was obtained as a gift sample from Ranbaxy Laboratories Pvt. Ltd. (Poanta Sahib, India).Methanol, ethanol, sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate dihydrate (AR grade) were obtained from different commercial suppliers.

Preparation of Cocrystals

Pharmaceutical cocrystals of efavirenz were prepared with different cocrystal formers using solvent drop grinding and solvothermal methods. EFA-OXA cocrystal was prepared by grinding efavirenz and oxalic acid dihydrate in 1:1 molar ratio in a pestle and mortar for 120 minutes with addition of a few drops of methanol (approximately 10% of weight). The solid powder was then scratched from walls of mortar and stored in vial. EFA-CITR was prepared by adding efavirenz and citric acid monohydrate in 1:1 molar ratio to 5 mL of ethanol and refluxing at 80 ºC for 3 hrs. The clear hot solution was then evaporated immediately under vacuum using rota-vapour until a dry solid powder was obtained that was scratched from the walls of flask and stored in vial. The solid obtained in both experiments were then characterized using various analytical techniques.

Characterization of cocrystals

Differential scanning calorimetry (DSC)

DSC thermograms of efavirenz, coformers & their cocrystals were obtained on DSC, Q20, TA instruments-Waters and LLC USA. The instrument was calibrated for temperature and heat flow accuracy using the melting of pure indium (mp156.6°C and ΔH of 25.45 Jg^{-1}). The temperature range was from 25-250°C with a heating rate of 5°C per minute.

Thermal gravimetic analysis (TGA)

The TGA scans were obtained on TGA, Mettler, Stare SW 9.00. TGA traces were recorded at heating rate of 10°C per minute under a nitrogen purge of 50 mL. Samples with masses between 1-10 mg were analyzed using aluminum pan. Mass loss (%) was calculated based on the mass of original sample.

X- ray powder diffraction (XRPD)

Powder diffraction patterns of efavirenz, coformers & their cocrystals were recorded on an X-ray diffractometer (XPERT-PRO,

Netherlands, Holland) with Cu as tube anode the diffractograms were recorded under following conditions: voltage 35 kV, 20 mA, angular range 5, divergence slit 10, receiving slit 0.15 mm.

Fourier transform infrared spectroscopy (FTIR)

The FT-IR spectra of efavirenz, coformers , physical mixture & their cocrystals were obtained on Spectrum RX I, FT-IR spectrometer, (Perkin Elmer, UK) over the range 400- 4000cm^{-1}. Dry KBr (50mg) was finely ground in mortar and samples (1-2mg) were subsequently added and gently mixed in order to avoid trituration of the crystals. A manual press was used to form the pellets.

Calorimetric study

Calorimetric studies were performed on Microreaction calorimeter obtained from Thermal Hazards Technology, UK. Phosphate buffer (pH 7.0) was used to determine the enthalpy of solution and the measurements were performed at 25 °C and 37 °C.The size of sample used in this study ranged from 1 to 10 mg and was weighed (Sartorius Model CP225D) into a cylindrical glass tube covered with parafilm on one side. This cylindrical tube was submerged into the ampoule containing the solvent. A plunger with a cap was put from the open end of the tube. The same solvent was put into the reference ampoule. These were put into the sample and reference holes until both rest on the base of the holes. The parafilm was shattered mechanically by means of plunger.

Equilibrium solubility study

Equilibrium solubility studies of efavirenz and its cocrystals were performed by introducing an excess amount of sample in phosphate buffer pH 7.0 which was shaken in water bath shaker (MSW-275 Macroscientific works, Delhi) at 37°C. The aliquots were withdrawn from the slurry at 0, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes. These were filtered through a 0.45 µm membrane filter paper (Millipore), diluted suitably and concentration determined by measuring the absorption at 245.2 nm using Perkin Elmer, Lambda 25, UV/VIS spectrophotometer. This was possible since none of the conformer absorbs at this wavelength and therefore did not interfere with the determination of concentration of efavirenz.

Concentration of drug was calculated using the formula

$$\text{Concentration of drug} = \frac{\text{absorbance} \times \text{dilution factor}}{\in \times b}$$

Where, Є is molar extinction coefficient & b is the path length.

Є = 1482 L.mole^{-1}cm^{-1}, b =1

RESULTS AND DISCUSSIONS

Differential scanning calorimetry (DSC) &thermogravimetic analysis (TGA)

DSC analysis has been shown to be a very useful thermoanalytical method in the characterization of the solid-state interactions between drug and coformers through the appearance, shifts or disappearance of endothermal or exothermal effects and/or variations in the relevant enthalpy. DSC experiments were carried out to study the thermal behavior of cocrystals with respect to the individual components. DSC thermograms of efavirenz, oxalic acid dihydrate, their respective physical mixture and their cocrystal are presented in fig 1.

The DSC thermogram of efavirenz shows sharp melting peak at 138.81°C while oxalic acid dihydrate shows a broad peak for desolvation of water molecule at 102.95°C followed by a sharp melting peak at 195.19°C (Table 1). However, the DSC scan of their physical mixture shows a large broad peak at 81.38°C followed by another quite broad peak at 133.97°C depicting that melting peak of both oxalic acid dihydrate and efavirenz have shifted to lower temperatures. These changes occurred as a consequence of interaction induced by thermal energy between the drug and the coformer, during the DSC scan of sample. The DSC thermogram for EFA-OXA cocrystal shows a broad peak at 95.11°C followed by a sharp melting peak at 121.83°C. This is different from that of starting components as well as from those appeared in DSC scan of physical mixture suggesting the formation of a new phase. TGA thermogram of EFA-OXA shows a weight loss of 4.26% in the range of 50-110°C which correspondes to a therotical weight loss of 4.76% due to evaporation of two water molecules from sample suggesting EFA-OXA to be adihydrate compound (Fig 2).

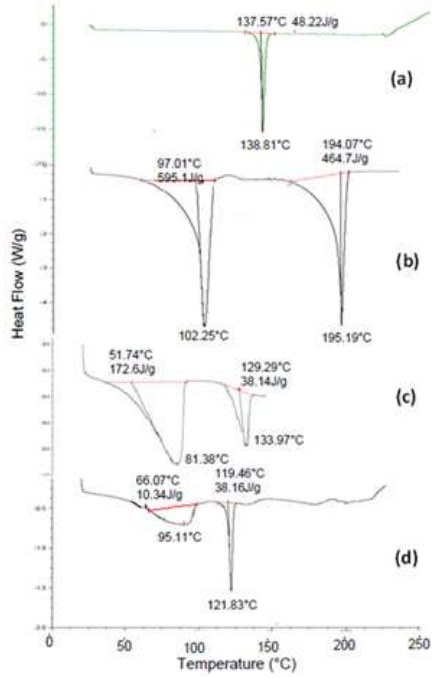

Fig. 1: DSC thermograms of (a) efavirenz (b) oxalic acid dehydrate (c) efavirenz - oxalic acid dihydrate (physical mixture) (d) EFA-OXA

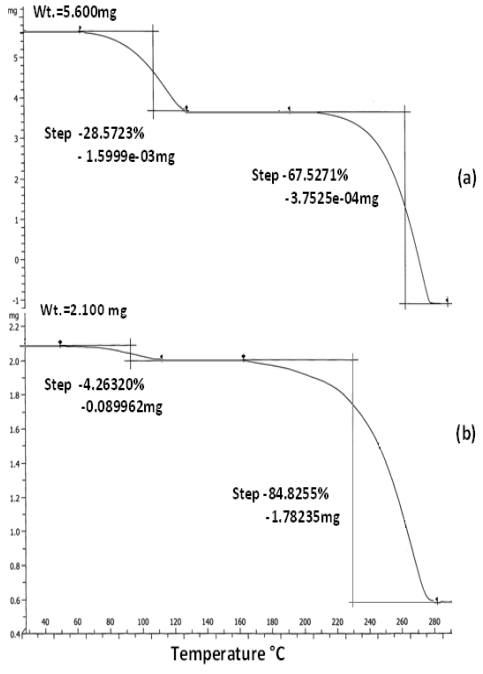

Fig. 2: TGA thermograms (a) oxalic acid dihydrate and (b) EFA-OXA

Table 1: Thermal characteristics of for efavirenz, oxalic acid, (efavirenz + oxalic acid) physical mixture, EFA-OXA, citric acid, (efavirenz + citric acid) physical mixture, EFA-CITR by differential scanning calorimetry

Sample	Desolvation Temperature (T_{des}) °C	Melting Point (T_m) °C
Efavirenz	--	138.81
Oxalic acid dihydrate	102.95	195.19
(Efavirenz + oxalic acid dihydrate) physical mixture	81.38	133.97
EFA-OXA	95.11	121.83
Citric acid monohydrate	56.49	155.59
(Efavirenz + citric acid monohydrate) physical mixture	52.23	134.98, 153.52
EFA-CITR	73.33	123.36

The DSC scan of citric acid monohydrate shows a broad peak due to desolvation of water molecule at 56.49°C followed by a melting peak at 155.59°C. The physical mixture of efavirenz and citric acid monohydrate shows a small desolvation peak at 52.23°C followed by melting peak of efavirenz at 134.98°C and citric acid at 153.52°C, while DSC scan of EFA-CITR shows a broad peak at 73.33°C depicting the desolvation of water molecule present in EFA-CITR, followed by a sharp melting peak at 123.36°C which is different from that observed in DSC scan of the physical mixture of the starting components indicating the formation of a new hydrous phase (Fig 3). Further TGA thermogram of EFA-CITR shows a weight loss of 2.28% in the range of 40-100°C which corresponds to a weight loss of 2.1% (theoretical mass loss) due to evaporation of one water molecule from sample suggesting EFA-CITR to be a monohydrate compound (Fig 4).

Thus, the DSC shows both EFA-OXA and EFA-CITR to be new hydrated phases which are further confirmed by XRPD and FT-IR analysis.

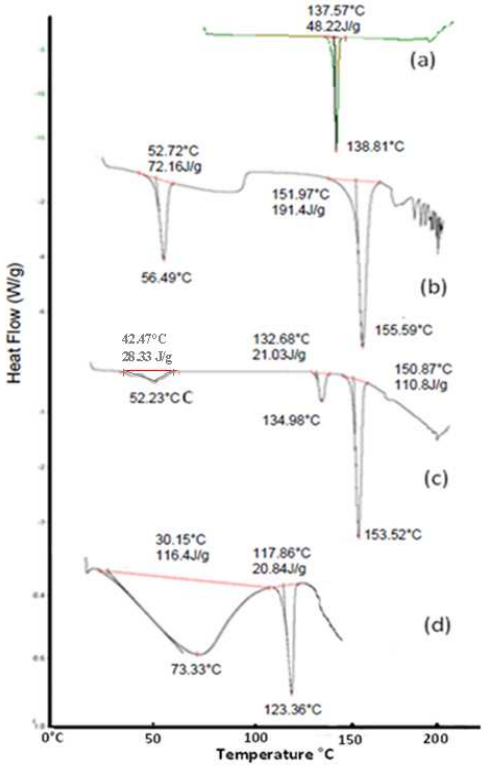

Fig. 3: DSC thermograms of (a) efavirenz (b) citric acid monohydrate (c) efavirenz- citric acid monohydrate (physical mixture) (d) EFA-CITR

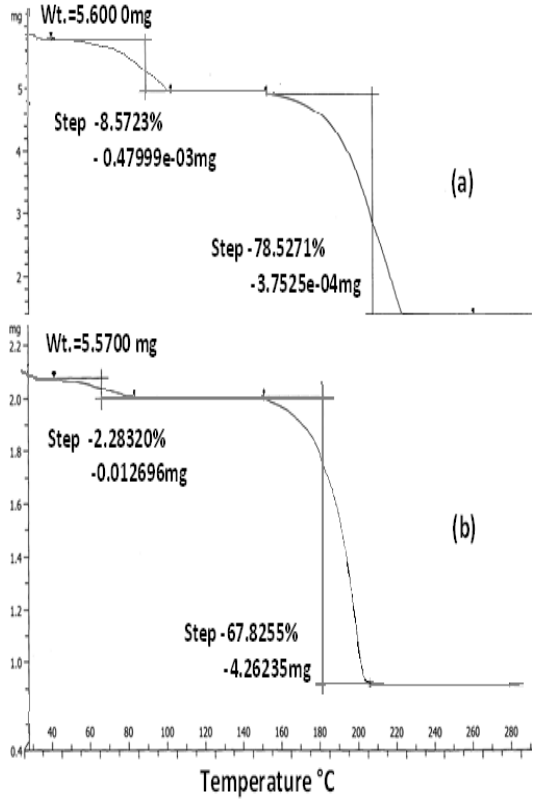

Fig. 4: TGA thermograms (a) citric acid monohydrate and (b) EFA-CITR

X- Ray powder diffraction (XRPD)

Powder XRD is a useful method for fast identification of the new phases. A different XRPD pattern for the cocrystals from those of the individual components confirms the formation of a new phase.

The XRPD patterns of efavirenz, oxalic acid dihydrate, citric acid monohydrate, and their respective cocrystals have been depicted in **fig 5 and6**.The XRPD pattern of efavirenz shows some characteristic peaks at 6.07°, 9.53°, 10.46°, 11.04°, 12.38°, 13.30°, 14.28°, 15.30°,16.92°, 19.32°, 20.15°, 21.36°, 21.86° 23.28°, 24.33°, 25.05°, 27.17°, 28.19°, 29.21°, 29.75° and 30.70 while the XRPD pattern of oxalic acid dihydrate shows characteristic peaks at 15.33°, 15.76°, 18.81°, 19.17°, 26.17°, 29.47°, 30.66°, 31.56°, 34.88° and 35.37°.

However, in XRPD pattern of EFA-OXA, certain characteristic peaks of efavirenz at 6.07°, 9.53°, 13.30°, 14.28° and 25.05° have disappeared and two unique peaks which are absent in both efavirenz and oxalic acid dihydrate have appeared at 12.06° (100% intensity) and 20.74° suggesting the formation of a new solid phase.

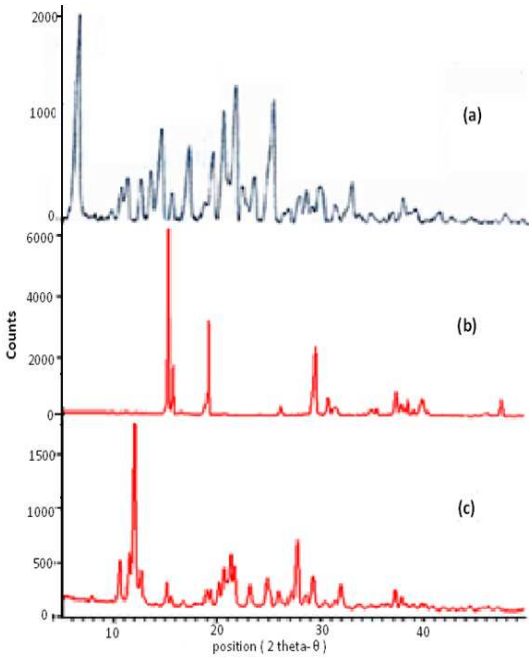

Fig. 5: XRPD pattern of (a) efavirenz (b) oxalic acid dihydrate (c) EFA-OXA.

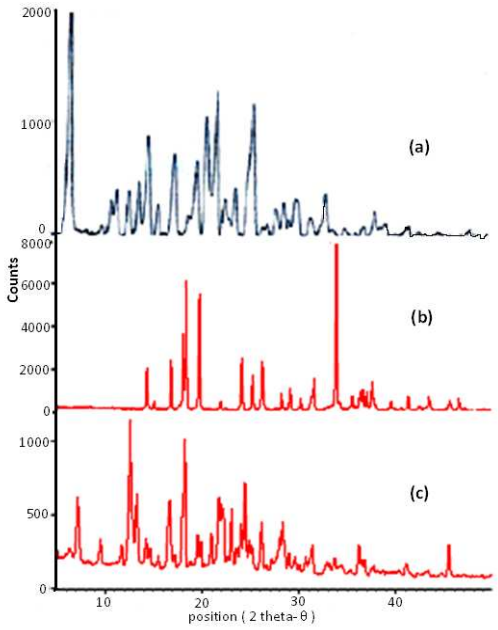

Fig. 6: XRPD pattern of (a) efavirenz (b) citric acid monhydrate (c) EFA-CITR.

XRPD pattern of citric acid monohydrate shows characteristics peaks at 14.31°, 15.04,16.80°, 18.04°,18.36, 19.72°, 21.89°,24.07°, 25.17°, 26.12°,26.27°,28.04°, 29.04°,30.13°,31.25°, 31.51°, 33.82°, 34.19°, 35.45°,36.17°, 36.29°, 36.54°, 36.93°, 37.46° and 39.41°. The XRPD pattern of EFA-CITR shows appearance of new peaks at 6.39°, 7.17°, 11.68°, 17.19°, 20.96° and 24.87° different from that of efavirenz and citric acid monohydrate. Besides this, characteristics peaks of efavirenz at (10.46° and 11.04°) and of citric acid at (33.83° and 35.45°) have disappeared in the XRPD pattern of EFA-CITR.

From the above results, it is clear that there is transformation in the crystalline lattices of efavirenz and coformers and new phases have been formed.

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR is an excellent technique to give an insight into the kind of interactions occurring between API and coformer. The FTIR spectra of efavirenz, oxalic acid dihydrate and citric acid monohydrate and their respective cocrystals are presented in fig 7 and 8.

The FTIR spectrum of efavirenz shows strong N-H stretch and C=O stretch of amide group at 3319.6 and 1749.7 cm^{-1} respectively, C-C stretch due to alkyne (C ≡ C) at 2250.7 cm^{-1}, C-F of $-CF_3$ at 1319 cm^{-1},1245 cm^{-1} and C-Cl stretch at 1038.3cm^{-1}.

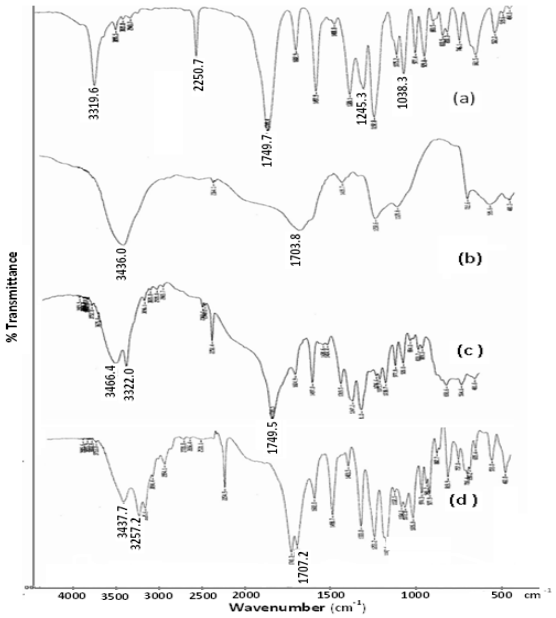

Fig. 7: FT-IR spectra of (a) efavirenz (b) oxalic acid dihydrate (c) efavirenz and oxalic acid dihydrate physical mixture and (d) EFA-OXA.

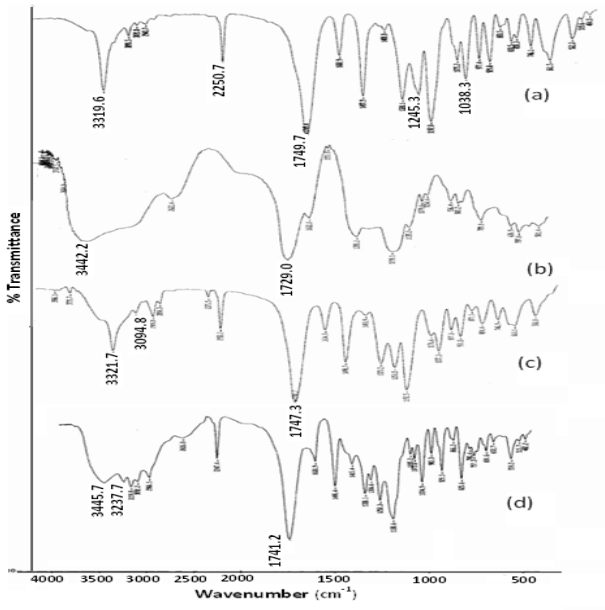

Fig. 8: FT-IR spectra of (a) efavirenz (b) citric acid monohydrate (c) efavirenz and citric acid monohydrate physical mixture and (d) EFA-CITR.

FT-IR spectra of oxalic acid dihydrate shows a broad peak at 3436.0 cm^{-1} due to O-H stretch of water molecule bound to acid and C=O stretch of carboxylic group at 1703.8 cm^{-1}. However, in FT-IR spectrum of EFA-OXA, N-H and C=O stretch of efavirenz have shifted to 3257.2 cm^{-1} and 1741.3 cm^{-1} respectively while C=O stretch of oxalic acid has shifted to 1707.2 cm^{-1}, suggesting that both N-H and C=O of amide group of efavirenz and C=O of carboxylic acid group of oxalic acid are participating in hydrogen bonding in EFA-OXA. Further, O-H stretch of water molecule bound to oxalic acid has also appeared in EFA-OXA without undergoing any significant shift i.e. at 3437.7 cm^{-1}, indicating that hydrate molecules of oxalic acid dihydrdrateare intact in EFA-OXA. Thus, combined with DSC results, it is concluded that EFA-OXA can be a cocrystal hydrate.

FT-IR spectra of citric acid monohydrate shows broad peak due to O-H stretch of the bound water molecule at 3442.2 cm^{-1} and C=O stretch of carboxylic group at 1729.0 cm^{-1}. However, in IR spectrum of EFA-CITR, N-H stretch of amide group of efavirenz have shifted to 3237.9 while C=O stretch of this amide group and C=O stretch of citric acid monohydrate have shifted to 1741.2cm^{-1} suggesting interaction between amide group of efavirenz and carboxylic acid group of citric acid monohydrate explaining the formation of a new phase. The O-H stretch of the bound water molecule of citric acid monohydrate appears at 3445.7 cm^{-1} in EFA-CITR indicating that this water molecule of coformer is also present in the newer phase suggesting it to be a cocrystal hydrate rather than anhydrous form. Thus, FT-IR supports the DSC results in that both EFA-OXA and EFA-CITR are multi component forms of efavirenz with oxalic acid dihydrate and citric acid monohydrate respectively as coformers.

Solution calorimetry

Calorimetrically determined ΔH_{sol} which depends upon the lattice energy and crystal structure has great potential in characterization of cocrystals. In cocrystals, complementary functional groups of two different molecules result in specific hydrogen bonding that is energetically more favourable than that between like molecules of either component. Hence, cocrystals are thermodynamically favored.

The ΔH_{sol} of individual components and their cocrystals was determined using phosphate buffer pH 7 at 37ºC. It is evident from the **table 2**, that oxalic acid dihydrate, citric acid monhydrate and both the cocrystals (EFA-OXA & EFA-CITR) show endothermic behavior whereas efavirenzshows exothermic behavior. The ΔH_{sol} of physical mixtures of efavirenz with oxalic acid dihydrate (1:1 ratio) and with citric acid monohydrate (1:1 ratio) have also been found to be endothermic in nature.

The ΔH_{sol} for cocrystals has been found to be more endothermic than the experimental and theoretically calculated (using equation 1) ΔH_{sol} of physical mixtures. This may be explained by the fact that in solution phase breaking of the hydrogen bonds (an endothermic process) is responsible for the increment in the endothermic effect of cocrystal. The excess ΔH_{sol} which is the measure of hydrogen bond between components gives an idea that cocrystals are sustained by strong hydrogen bonds.

$\Delta H_{(CAL)} = 0.5 \text{ X } (\Delta H_D) + 0.5 \text{ X } (\Delta H_{CCF})$ ..... Eq (1)

$\Delta H_{(CAL)}$ = Calculated molar enthalpy of solution of physical mixture

ΔH_D = Molar enthalpy of solution of drug

$\Delta H_{(CCF)}$ = Molar enthalpy of solution of cocrystal former

Table 2: Enthalpy of solution of drug, coformers, cocrystals and their physical mixtures

Sample	Enthalpy of solution (ΔH_{sol}) kJ/mol
Efavirenz	-9.24
Oxalic acid dihydrate	34.59
Efavirenz + oxalic acid dihydrate (physical mixture)	15.10 (Theoretically calculated = 12.67)
EFA-OXA	27.48
Citric acid monohydrate	27.60
Efavirenz + citric acid monohydrate (physical mixture)	9.25 (Theoretically calculated = 9.54)
EFA-CITR	19.66

The fig 9 (a) demonstrates that in EFA-OXA, the API (efavirenz) and oxalic acid dihydrate is in (1:1) molar ratio where oxalic acid dihydrate is attached to the API through two hydrogen bonds which are formed between of N–H of cyclic amide group of efavirenz and C=O of carboxyl group of oxalic acid dihydrate and another hydrogen bond is generated between C=O of amide group of efavirenz and of O–H of carboxyl group of oxalic acid dihydrate.The two molecules of water of crystallization are attached to one molecule of oxalic acid through hydrogen bonding between OH- of COOH- group & oxygen of water molecules.

Similarly in EFA-CITR, fig 9 (b) API is bonded to citric acid monohydrate in (1:1) molar ratio.One hydrogen bonds is formed between -N–H of amide group of efavirenz and -C=O of carboxyl group of citric acid monohydrate, whereas, another hydrogen bond is formed between -OH of carboxyl group of citric acid monohydrate and -C=O of amide group of efavirenz. One molecule of water of crystallization is linked to citric acid by hydrogen bonding between one of the hydroxyl group of citric acid and oxygen atom of water molecule.

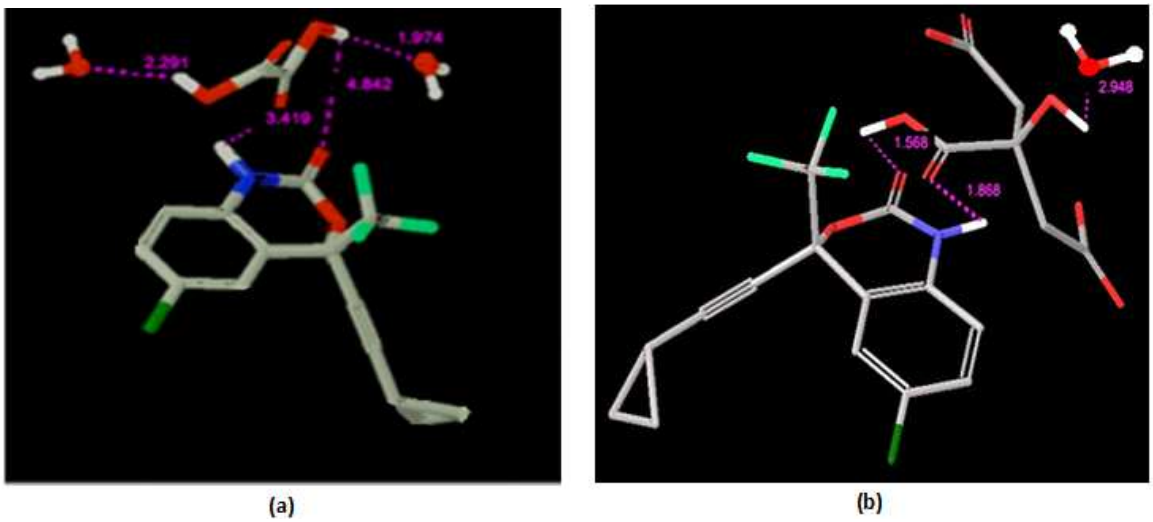

Fig. 9: A wireframe representation of (a) EFA-OXA and (b) EFA-CITR demonstrating the purposed hydrogen bonding between API and conformer.

Equilibrium solubility studies

Solubility studies of efavirenz and its cocrystals with oxalic acid dihydrate and citric acid monohydrate were carried out using phosphate buffer pH 7 at 37 ºC and results are given in table 3.Unless preliminary experiments have been performed, it is not known if equilibrium has been reached in the time frame used. Thus, for equilibrium solubility, a number of time points and measurements are taken to ensure that the solution has reached equilibrium as evidenced by a plateau in the concentration data (Fig 10).

Table 3: Equilibrium solubility studies of efavirenz and its cocrystals in phosphate buffer at pH 7

Time (in minutes)	Solubility of commercial sample (µg/ml)	Solubility of drug in EFA-OXA cocrystal (µg/ml)	Solubility of drug in EFA-CITR cocrystal (µg/ml)
0	0	0	0
15	1	5	9
30	4	7	12
45	6	10	18
60	8	13	21
90	8	16	25
120	10	18	27
180	10	18	27
240	10	18	27
360	10	18	27

Efavirenz shows a steady increase in solubility that reached the equilibrium in about 2 h at 10 µg/ml. Solubility profile of EFA-OXA and EFA-CITR shows an initial increase of 1.8 and 2.7 times respectively in concentration of efavirenz as compared to commercial sample of efavirenz followed by a steady increase that reached the equilibrium in 2 h. The solid residues obtained after the solubility studies were then characterized by XRPD. The XRPD patterns of commercial sample of efavirenz as well as both the multicomponent forms were found to be similar to that of their original forms indicating both these cocrystals to be stable even after 6 hours of solubility studies in aqueous media (Fig11and 12).

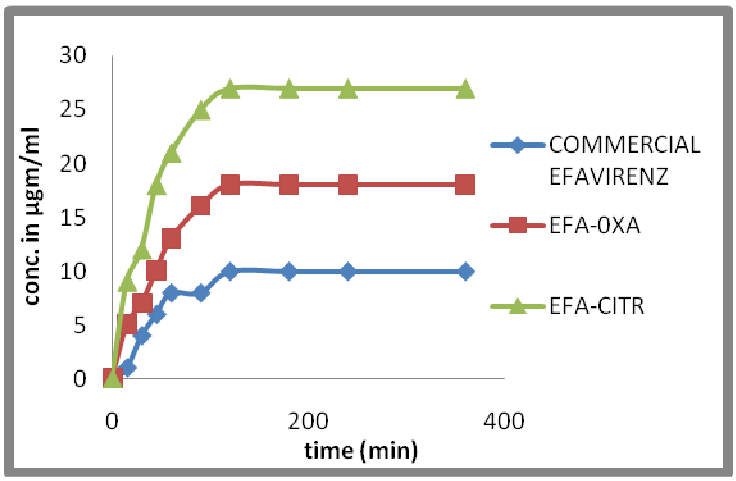

Fig. 10: Comparative equilibrium solubility profiles of drug and its cocrystals

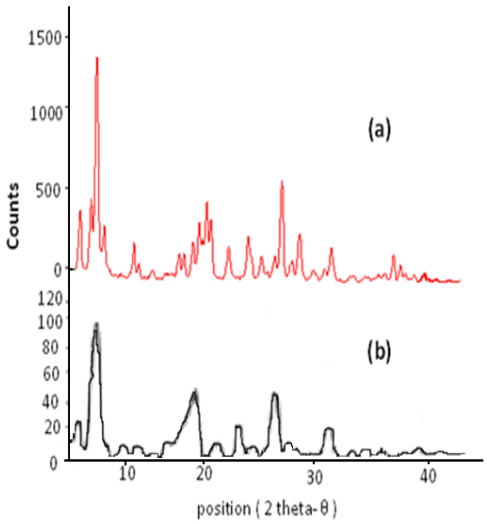

Fig. 11: XRPD pattern of (a) EFA-OXA before solubility studies (b) EFA-OXA after solubility studies inphosphate buffer pH 7 for 6 hours.

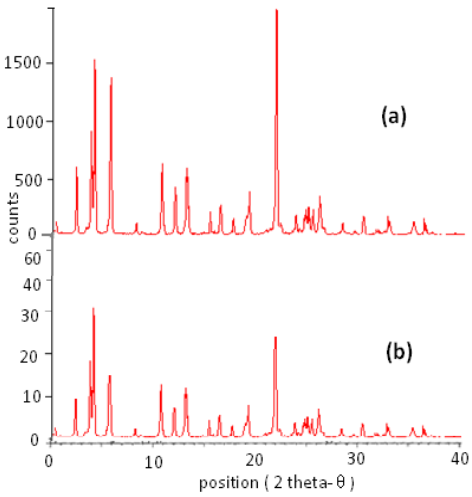

Fig. 12: XRPD pattern of (a) EFA-CITR before solubility (b) EFA-CITR after solubility studies in phosphate buffer pH 7 for 6 hours.

CONCLUSION

To further enhance the solubility of efavirenz, its cocrystals were prepared with oxalic acid dihydrate & citric acid monohydrate as coformers (GRAS status) using solvent drop grinding method & crystallization by fast evaporation from solvent under reduced pressure. Formation of new crystalline phase was indicated by DSC thermogrms which exhibited a single sharp melting endotherm at a position different from that of efavirenz and coformers. Presence of broad desolvation endotherm before the true melting, which is accompanied by mass loss in TGA, indicated the formation of solvated cocrystals. XRPD studies revealed these cocrystals to be different crystalline identity from the API and conformer used. Increased endothermic behavior of enthalpy of solution of both the cocrystals compared to data obtained experimentally and calculated for physical mixture indicates the involvement of hydrogen bonds in the formation of cocrystals. Equilibrium solubility studies showed 1.8 & 2.7 fold enhancement solubility of the cocrystals of EFA-OXA & EFA-CITR respectively.

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