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Original Article

Co-crystals: A Novel Approach to Improve the Solubility of Apixaban

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ABSTRACT

Objectives: The current study work was aimed to improve the solubility of Apixaban by forming co-crystals. **Materials and Methods:** Two solvents mainly Dimethyl Sulphoxide (DMSO) and 2,2,2-trifluoroethanol were tried during the formulation of co-crystals. The other chemicals oxalic acid, adipic acid and L-Tartaric acid were used. **Results:** From the different trials it was concluded that oxalic acid and DMSO combination with the drug gave the better results for improvement in the solubility. Hence the batch F3 was analysed further. The melting point of F3 was found lower than pure drug. Drug content was found optimum as 95.06 ±0.65%. FTIR-spectra demonstrated the combined peaks of drug and oxalic acid. DSC thermogram showed the sharp endothermic peak similar to pure drug. SEM and XRD spectra confirmed the formation of co-crystals. **Conclusion:** It was found that formation of co-crystals of apixaban and oxalic acid was satisfactory and it was beneficial to improve the solubility of the apixaban.

Keywords: Apixaban, Co-crystal, Solubility, Oxalic acid.

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INTRODUCTION

Solubility and rate of dissolution rate play an important role in absorption, bioavailability and in turn efficacy of any drug. Poor solubility and rate of dissolution limit the applications of various efficacious drug molecules. Enhancement of the solubility and improvement of the dissolution profile of poorly soluble drugs without changing their molecular structure has become one of the main current challenges in the pharmaceutical industry.[1] Till now, several approaches have been implemented for improvement of the aqueous solubility of drugs including salt formation, emulsification, micronisation, solubilisation through co-solvents and by using of polymer drug vehicles for the delivery of poorly soluble drugs. The accomplishment of these approaches is totally dependent on the particular physico-chemical properties of the molecules being studied.[2] Over last few years there has been an enhanced interest in the design and development of pharmaceutical cocrystals emerging as a potential method for enhancing the bioavailability of drugs with low aqueous solubility.

Cocrystals are the homogeneous crystalline materials composed of two or more components, out of which one component is an Active Pharmaceutical Ingredient (API) and others are conformers such as pharmaceutical excipients, preservatives, additives etc. They are present in stoichiometric amount and are distinct neutral molecular reactants which are in the solid at ambient temperature.[3] Co-crystals possesses several advantages such as no requirement of making or breaking of covalent bonds, stability of crystalline form, cocrystalinity of all type API molecules and presence of several conformers, improved physico-chemical and pharmacokinetic properties of an API without altering its pharmacodynamic activity.

Apixaban is a direct inhibitor of blood clotting factor Xa (FXa) that has been approved in many countries for several indications.[1,2] It is poorly soluble in water (0.028 mg/mL at 24°C) and possesses a lower bioavailability (about 50% for a single 10 mg dose).[4] The aim of the present research work is to improve the solubility of the Apixaban by using co-crystal approach. The co-crystals were developed by using selected carboxylic acids mainly, oxalic acid, adipic acid and L-Tartaric acid. The co-crystals were developed by using dimethyl sulphoxide and 2,2,2-trifluoroethanol as solvents and improvement in solubility was evaluated.

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Table 1: Different batches of co-crystals of Apixaben.

Batch No.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Apixaban	459	459	459	459	459	459	459	459	459	459	459	459
Oxalic acid	90	90	180	180	-	-	-	-	-	-	-	-
Adipic acid	-	-	-	-	146	146	292	292	-	-	-	-
L-Tartaric acid	-	-	-	-	-	-	-	-	150	150	300	300
DMSO	5	-	5	-	5	-	5	-	5	-	5	-
2,2,2-trifluoroethanol	-	5	-	5	-	5	-	5	-	5	-	5
Ethyl acetate	10	10	10	10	10	10	10	10	10	10	10	10

MATERIALS AND METHODS

Materials

Apixaban was procured as a gift sample from Dr. Reddy's Laboratories Limited, Hyderabad, India. HPMC E50 LV, HPMC E5, PEG 6000, PVP K-30 were purchased from SD Fine Chemicals Limited, Mumbai. All the other solvents and reagents used were of analytical grade.

Methods

Formulation of co-crystals

The co-crystals of apixaban were prepared by the method used with certain modification. A mixture of apixaban and anhydrous acids mainly oxalic acid, adipic acid and L-Tartaric acid in Dimethyl Sulphoxide (DMSO) was agitated at room temperature for 12 hr. To this mixture ethyl acetate was added dropwise to form a precipitate. The precipitate was filtered and dried at 60°C for 12 hr by application of vacuum. The filtrate was transferred in the closed glass desiccator comprising phosphorous penta oxide and allowed to vaporize. After 1 week, block-design crystals of apixaban-Oxalic acid were formed.[5] Resulting product was stored in the desiccator for till further use. Different batches were prepared by varying the solvents and concentration of acid at two levels as shown in Table 1.

Evaluation of co-crystals

Solubility

The excess amount of co-crystals was added in 5 mL of water, sonicated for 1 hr, and stirred with the help of thermostatically maintained shaker at a temperature kept at 35°C for 24 hr. The solution was sifted through a 0.45µm filter and diluted with purified water and analysed using a UV-spectrophotometer at 278nm (Shimadzu 1800).[1,2]

Drug content

The percent drug content of apixaban co-crystals was estimated by dissolving co-crystals equivalent to 10 mg of apixaban in 50 mL DMSO. The samples were sonicated for 15 min and filtered through the Whatman filter paper 0.45µm and again diluted 5 mL filtrate to 100 mL with methanol. The absorbance of the sample was taken at 278nm.[1,2]

The drug content was determined by the formula:

Drug content (%) = Weight of drug in co-crystal/ Total weight of sample X 100

Melting point determination

The melting points of co-crystals were evaluated by the capillary method. Fine powders of co-crystals were filled in a glass capillary tube (formerly closed on a single side). The capillary tube was inserted in Thiele tube containing liquid paraffin and side tube was heated. The melting temperature range of the crystals were noted.[6]

Fourier transform infrared spectroscopy (FTIR)

Co-crystals were mixed with potassium bromide and the FTIR-spectra of sample was recorded using FT-R-spectrophotometer (Shimadzu, Japan) within the operating range of 4000-400 cm^{-1}.[7]

Differential scanning calorimetry (DSC)

Sample about 2 mg was placed in aluminum pans and heated at 10°C/ min over a temperature range from 10 to 300°C under dry nitrogen atmosphere (with a flow rate of 100 mL/min) using DSC device (Mettler Toledo DSC 8-32-3).[8]

X-ray diffraction (XRD)

The XRD was performed by using Diffract plus XRD D8 and the information was measured with an angular range from 0 to 60 diffraction angle (2θ) in uninterrupted image style employing a phase size of 0.100 2θ and a time of 0.1 sec.[9]

Microscopy

Microscopy of co-crystals were investigated by Motic microscope.

Surface morphology

Surface morphology was studied by scanning electron microscopy (SEM). The sample was fixed on support and coated with gold in

Table 2: Solubility data of different batches.

Batch no.	Solubility (µg/mL)
F1	8.36 ± 0.55
F2	6.30 ± 0.87
F3	8.58 ± 0.67
F4	6.03 ± 0.38
F5	4.45 ± 0.76
F6	4.44 ± 0.93
F7	4.00 ± 0.43
F8	3.90 ± 0.79
F9	3.10 ± 0.38
F10	3.00 ± 0.59
F11	3.05 ± 0.53
F12	2.90 ± 0.86

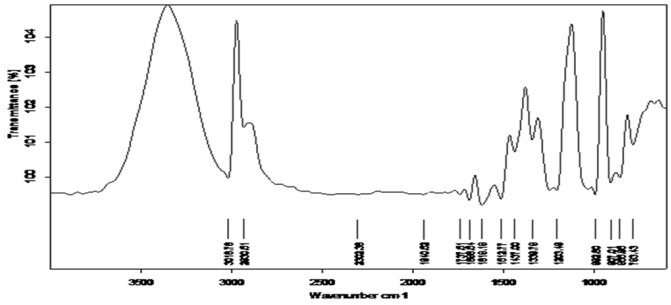

Figure 1: FTIR-spectra of apixaban-oxalic acid co-crystals.

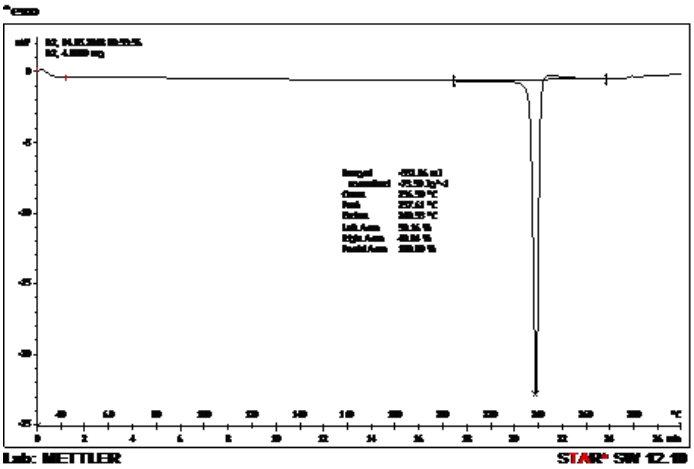

Figure 2: DSC thermogram of apixaban-oxalic acid co-crystals.

a high vacuum evaporator. Prepared sample was observed with scanning electron microscope (JEOL JSM-6360A, Japan).[10]

RESULTS

Co-crystals of apixaban in combination with adipic acid, oxalic acid and L-tartaric acid was prepared to improve the solubility of apixaban. 1:1 and 1:2 ratios of drug and acid were evaluated to improve the solubility. The effect of solvents DMSO and 2, 2, 2-trifluoroethanol was also tested. The co-crystals are mainly prepared with the aim to improve the solubility. The effect on these parameters on solubility was shown in Table 2. The results of the solubility indicated that formulation containing 180 mg of oxalic acid (F3) showed the maximum solubility. The formulation prepared with L-tartaric acid showed least solubility.

The melting point of the pure drug was 235°C and for formulation it was 220°C, while meting point of oxalic acid was 101.2°C. It was hypothesized that the melting point of co-crystal fell between melting point of drug and conformer.[11] It was also observed that there was correlation between solubility and melting point. Lower melting point resulted in good solubility.[3]

FTIR of co-crystal apixaban showed O–H stretch of oxalic acid at 3016.78 and 783.43, 1213.48 showed CH_2 wagging, C=O stretch of primary amide at 1612.11, lactam at 1818.18 and 1599 for N=H bending. The FTIR-spectra of apixaban-oxalic acid co-crystals was shown in Figure 1.

DSC thermogram of apixaban-oxalic acid co-crystal (F3) was shown in Figure 2. It showed sharp endothermic peak onsets from 236.30°C and ends at 240.53°C. This endotherm resembles the apixaban melting point. These results were in agreement with the research carried out.[12] It shows the crystalline nature of apixaban-oxalic acid co-crystal.

The XRD of Apixaban-Oxalic acid co-crystal (F3) was performed by using the Diffractometer D8. The 2θ data of diffraction angle was accumulated over an angular range from 0 to 60° in an uninterrupted scan manner with a step size of 0.10 2θ and a time of 0.1sec. The XRD graph was shown in the below Figure 3. The various sharp peaks indicative of formation of pure crystal structure. This result was in agreement with the studies carried out.[5]

Microscopy of co-crystals were investigated by Motic microscope. The crystal possesses square shape structure as shown in (Figure 4).

Surface microscopic characteristics of the crystals was studied by SEM, it also confirms formation of square shaped crystals. Surface of the crystal was found smooth as shown in Figure 5.

DISCUSSION

Cocrystals formers are capable of co-crystallizing with drug. Most pharmaceutically acceptable Co-crystal formers are carboxylic acids, carbohydrates, amides, amino acids and alcohols. Carboxylic acids possess self-complementary hydrogen bond donor and acceptor as C=O and H–O group. Apixaban contains three amide groups, and the molecules are linked together through a N–HO intermolecular hydrogen bond to form a one-dimensional chain and it may interact with carboxylic acid through hydrogen bonds. Hence in the current study carboxylic acids were selected as conformer. Formulations with adipic acid showed intermediate results between oxalic acid and L-tartaric

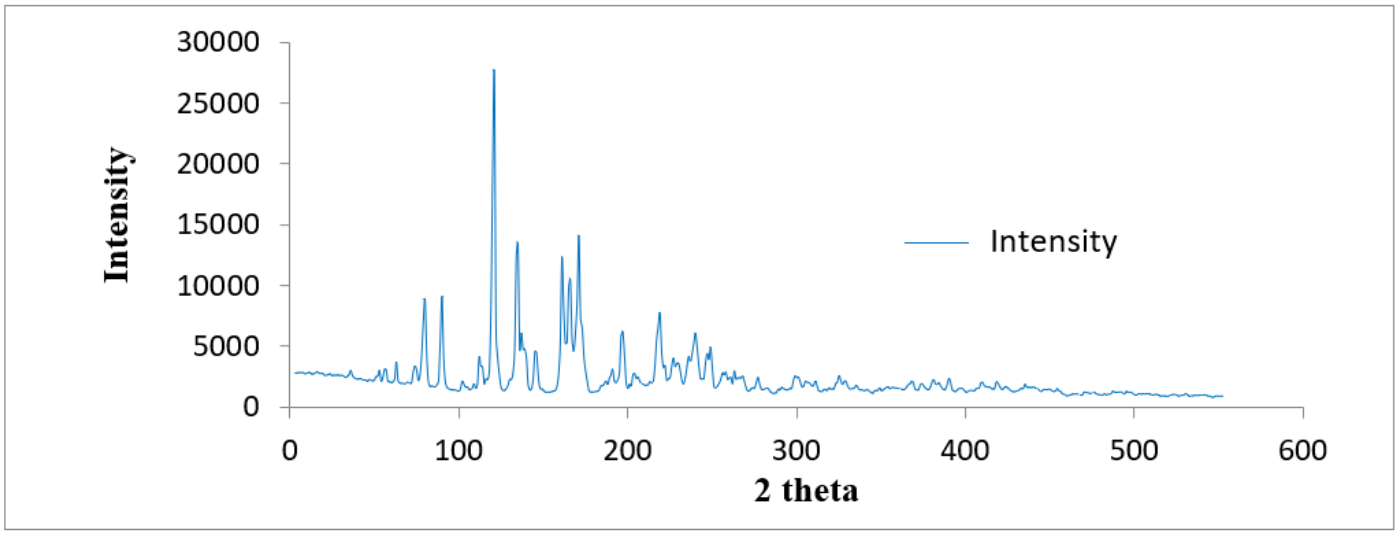

Figure 3: XRD graph of Apixaban-Oxalic acid co-crystals (F3).

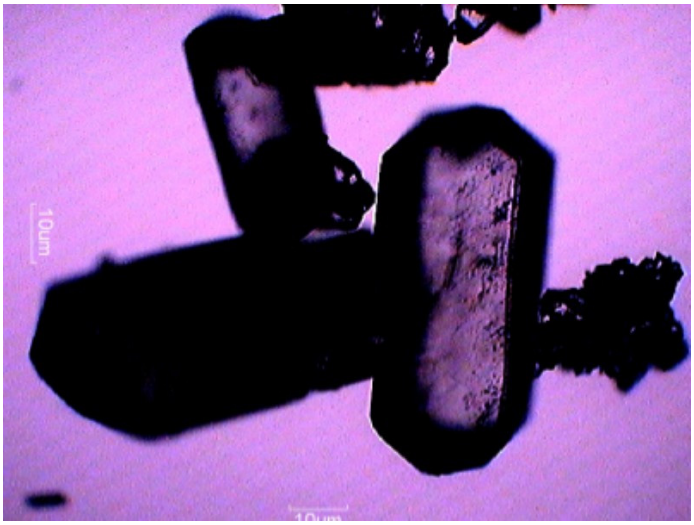

Figure 4: Motic microscope image of Apixaban-Oxalic acid co-crystals (F3).

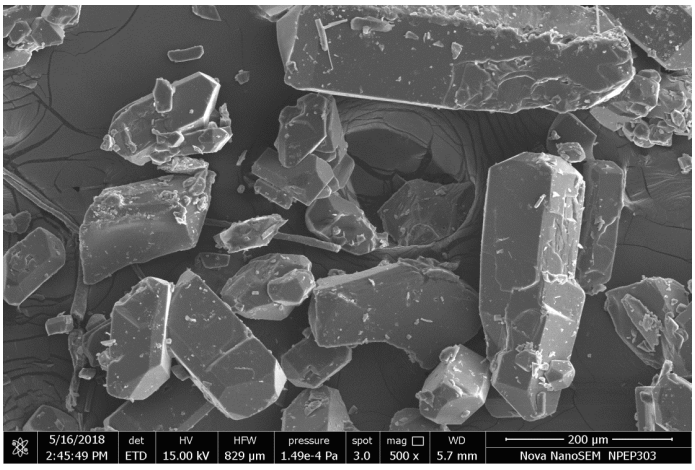

Figure 5: SEM image of Apixaban-Oxalic acid co-crystals (F3).

acid. These results might be due to formation of hydrogen bonds with oxalic acid and bond formation is less favoured in adipic and L-tartaric acid. Hence batch F3 was considered as optimized formulation and further tests were carried on batch F3. The evaluated drug content of selected apixaban-oxalic acid co-crystals batch no. F3 was observed at 95.06% ±0.65. This result indicates of good entrapment efficiency. Further FTIR and DSC thermogram studied were performed and these studies confirmed the crystalline structure of co-crystals. The square shape crystal morphology of F3 was confirmed by XRD and SEM studies, respectively.

CONCLUSION

Apixaban has been suffering from the drawback of poor solubility. A novel approach has been tried to improve the solubility of Apixaban by developing the co-crystals. Co-crystal of oxalic acid; adipic acid, or L-tartaric acid with solvents mainly DMSO and 2, 2, 2 trifluoroethanol were developed. Oxalic acid co-crystal (batch no. F3) in the ratio 1:2 (Apixaban: oxalic acid) by using DMSO as solvent resulted in highest improvement in the solubility of 8.58 ± 0.67 µg/mL. This increment was approximately 3 fold more than solubility of plain drug. The selected co-crystal formulation showed decreased melting point from parent drug. The FTIR-spectra resulted in a combination peak of apixaban and oxalic acid. DSC study confirms a sharp endotherm peak resembles the melting point of pure apixaban. The crystalline nature was further confirmed by XRD and microscopic studies. SEM image showed smooth square shaped crystalline structure. The formation of cocrystal of apixaban-oxalic acid was confirmed and was found effective to enhance the solubility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

API: Active pharmaceutical ingredient**; HPMC:** Hydroxy Propyl Methyl cellulose**; PVP:** Polyvinyl pyrolidone; **DMSO:** Dimethyl sulphoxide; **FTIR:** Fourier transform infrared spectroscopy; **DSC:** Differential scanning calorimetry; **XRD:** X-ray diffraction; **SEM:** Scanning electron microscopy.

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