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Original Article

PREPARATION, SOLID STATE CHARACTERISATION OF PACLITAXEL AND NARINGEN COCRYSTALS WITH IMPROVED SOLUBILITY

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ABSTRACT

Objective: The objective of the present study is to prepare a better form of paclitaxel cocrystal with improved solubility. Paclitaxel (PTX) is a class-4 drug; this drug has low aqueous solubility and high affinity for P-gp. Available formulation are IV based and using our research work with advantages of co-crystal technology towards the enhancement of paclitaxel solubility and thereby its bioavailability (1) and also to improve the patient compliance.

Methods: Naringen was selected based on their chemical nature and its ability to inhibit P-gp, solvent assisted grinding method used to prepare the cocrystals, and prepared cocrystals were subjected to solid state characterization to determine the crystal structure of the cocrystals, as this can provide significant new insights into how the drug and coformer interact, and thereby provide an excellent crystal engineering guide to new cocrystals, potentially with improved properties. Instruments like Fourier transform infrared spectroscopy(FTIR), differential scanning calorimetry, X-ray powder diffraction will be used to determine their stability and any phase transformations (including decomposition) which they might undergo as a function of temperature.

Results: Principle involved in the formation of cocrystal is hydrogen bonding between C=O and N-H group of drug and COOH groups of coformers, which is confirmed by FTIR data and DSC experiments were carried out to study the melting point and heat of enthalpy of the cocrystals. Results clearly shows that the melting point of the cocrystals was increased which confirms the formation of cocrystals. The drug and formation of cocrystals are explained by the X-ray powder diffraction patterns. The PXRD patterns of the pure drug showed sharp, well-defined peaks(spectrum attached) and cocrystals PXRD patterns shows that there is a significant difference in the entire diffraction pattern, changes in peak locations with respect to pure drug indicates a change in the arrangement of molecules, hence confirms the development of new crystalline phase.

Conclusion: The results obtained from the above experiments clearly shows the formation of cocrystals with improved solubility.

Keywords: Paclitaxel, Cocrystals, Solid state characterization, Naringin

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INTRODUCTION

In the pharmaceutical industry, less than 1% of pharmaceutically active compounds eventually appear in the marketplace mainly due to its poor biopharmaceutical properties rather than toxicity or lack of efficacy. The solubility remains the key issue among these biopharmaceutical properties. According to recent studies, 75% of the drug development candidates had low solubility and belonged to biopharmaceutical classification system (BCS) classes II and IV.

Many approaches like micronisation, salt formation, emulsification, solubilisations using co-solvents, use of polymer drug vehicles for delivery of poorly soluble drugs, reduction in the particle size (nano–drug delivery) have been used to improve the aqueous solubility of drugs. Although these techniques have been shown to be effective at enhancing oral bioavailability, the success of these approaches is dependent on the specific physicochemical nature of the molecules being studied. Over the last decade, design of pharmaceutical cocrystals emerged as an important method for improving the bioavailability of drugs with low aqueous solubility [1].

Cocrystals are structurally homogeneous crystalline materials containing two or more components (generally referred to as drug and coformer) indefinite stoichiometric amounts [1]. Cocrystals are different from other multicomponent crystals such as hydrates or salts in a manner that drug and coformer are solids at ambient temperature and that the intermolecular interactions are non-ionic in nature [2].

Pharmaceutically pertinent properties that can be affected by cocrystallization include but are not limited to solubility, dissolution, moisture uptake, chemical stability, mechanical properties, and bioavailability. Of these properties, solubility is the most widely appreciated in the literature [2].

Paclitaxel is a drug of choice in the treatment of breast cancer, it has a bioavailability at less than 10% because of limited aqueous solubility, also affinity towards CYP metabolic enzymes and the P-glycoprotein [4]. Many attempts were made to improve the oral bioavailability of paclitaxel-like selective modulation of P-glycoprotein, self-emulsifying drug delivery systems and paclitaxel-loaded lipid nanocapsules [5]. Cocrystals technique can be used as one of the technique because of it commercial feasibility and also for increase the diversity of solid-state forms of a drug even for non-ionizable drugs, and enhance pharmaceutical properties by modification of chemical stability, moisture uptake, mechanical behaviour, solubility, dissolution rate, and bioavailability [6, 7].

Oral treatment with anticancer agents is, if feasible, to be preferred, as this route of administration is convenient to patients, reduces administration costs and facilitates the use of more chronic treatment regimens. There are some efforts for enhancing the oral bioavailability of paclitaxel by d-alpha-tocopheryl polyethylene glycol 400 succinate in mice and results indicated that TPGS 400 enhances the oral bioavailability of paclitaxel in mice and the enhancement may result from an increase in intestinal absorption of paclitaxel [8].

Paclitaxel cocrystals were prepared using solvent assisted co-grinding and co-grinding method and with the help of solid-state characterization, paclitaxel with tartaric acid, succinic acid and nicotinamide in the molar ratios of 1:1 using co-grinding method was confirmed the formation of cocrystals [9].

Naringin is one of the widely available natural flavonoids; it inhibits cytochrome enzymes. So we have selected naringin as one of the coformer because it has two major advantages as coformer i.e. it inhibits cytochrome enzymes so we can prevent the metabolism of paclitaxel by using this flavonoid and also it easily forms cocrystal

with paclitaxel based on the structural configuration so we can expect improvement in the solubility as well as bioavailability of the paclitaxel cocrystals. Using naringin as coformer we are going to develop paclitaxel cocrystals with enhanced solubility while maintaining or improving other relevant physical properties (stability, processability, etc.). and always oral treatment with anticancer agents is, if feasible, to be preferred, as this route of administration is convenient to patients, reduces administration costs and facilitates the use of more chronic treatment regimens.

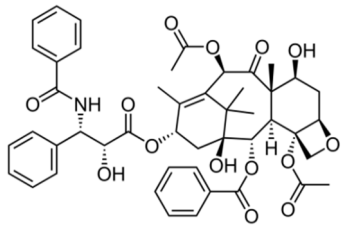

Fig. 1: Chemical structure of Paclitaxel

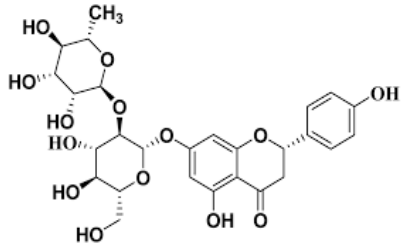

Fig. 2: Chemical structure of Naringin

MATERIALS AND METHODS

Materials

Drug: Paclitaxel as gift sample from Cipla, Mumbai and Intas, Mumbai.

Coforms: Naringin from sigma Aldrich.

Acetonitrile (HPLC grade) and Triethylamine: Merck Life Sciences Private Limited, Mumbai.

Orthophosphoric acid: Nice chemicals, Mumbai.

Instruments used: High performance liquid chromatography (HPLC) from Shimadzu LC-10 series chromatographic system, Shimadzu Corporation, Kyoto, Japan., Differential Scanning calorimeter (DSC) from Shimadzu, DSC-60, Japan., Fourier transform infrared spectroscopy (FTIR) from Shimadzu FTIR-8300 system, Kyoto, Japan., Powder X-ray diffractometer (PXRD) from Rigaku mini flex 600, Rigaku Co., Tokyo, Japan., Incubator shaker from Labtop Instruments Pvt. Ltd, Maharashtra, India.

Methods

Selection of coformers

The selection of relevant cocrystal former becomes a crucial issue and requires creating supramolecular libraries of crystallizing agents. Analysis of existing crystal structures represents the first step in a crystal engineering experiment and thereby collecting the information concerning common functional groups and how they engage in the molecular association. Carboxylic acid moieties represent one of the most commonly studied functional groups in crystal engineering These are excellent starting point for crystal engineering of pharmaceutical co-crystals and the complementary hydrogen bond donor, and acceptor sites make them more favorable coformers, but the selection of coformers not limited to carboxylic acids, the alcohol amine, alcohol pyridine supramolecular hetero synthons are also well established in crystal engineering.

Coformer with higher solubility tends to increase cocrystal solubility effectively, hence selection of conformer plays a crucial role.

A search of the cambridge structural database will be employed to identify likely hydrogen-bonding motifs for paclitaxel. Synthesis of new co-crystal materials will be guided by the outputs of this work.

Naringin was selected as the coformer because of its various advantages listed in the introduction.

Preparation of cocrystals of paclitaxel: naringin

Different Mole ratios of paclitaxel and naringin were weighed separately mainly 1:1, 1:2, 2:1 molar ratios. Different ratios of the drug and the individual coformers were subjected to optimized solvent assisted solid state grinding method and triturated at a constant speed without producing heat for about 30 min using a granite mortar and pestle [7, 11]. The resulting powder mixture was collected, stored for analysis. The batches with the mole ratios are mentioned in table 1. The powder samples were then collected for further (PXRD, FTIR and DSC) analysis.

Table 1: Batches of paclitaxel cocrystals prepared using co-grinding method

S. No.	Co-crystal batch code	Co former used	Technique used	Ratio
1.	CCN-1	Naringin	Solvent assisted Co-grinding	1:1
2.	CCN-2	Naringin	Solvent assisted Co-grinding	1:2
3.	CCN-3	Naringin	Solvent assisted Co-grinding	2:1

Stability studies

Drug content determination of cocrystals and chemical stability

Chemical stability was carried out by dissolving a known amount of sample in the mobile phase and injected the sample to HPLC equipped with photodiode array detector. The total peak purity was calculated for the sample peak to confirm that no coelution from the degradants to the peak of interest with the help three-dimensional assessment of the chromatogram with the help of LC solution software and drug content was calculated with reference to initial assay value.

Physical stability

Optimized CCN-1 batch was stored under dry conditions in desiccators (dry condition was achieved by using $CaCO_3$ crystals at the bottom of the desiccator) at 25 ° and 40 °C for up to 30 d and further extended up to 90 d. The samples were analyzed by XRPD.

Solubility studies of prepared cocrystals

To evaluate the enhancement of solubility of a crystalline form, it is important to investigate the saturation solubility. Equilibrium solubility of paclitaxel cocrystal shall be determined at room temperature by shake flask method over 72 h.

An excess amount of pure, physical mixture and cocrystals of Paclitaxel were added separately to ultra-clear water and phosphate buffer pH 6.8. Then the mixture was kept in an orbital shaker (100 agitations/min) for 72 h at room temperature.

The samples were filtered through a 0.45 µm membrane filter (Millex-HA filter units, Millipore) and suitably diluted with diluent before analysis. The samples were analyzed by HPLC method as described below. The experiments were conducted in triplicates (n=3).

Analytical techniques

HPLC analysis for saturation solubility and drug content determination and chemical stability

The analysis was carried out on a shimadzu LC-10 series chromatographic system (Shimadzu Corporation, Kyoto, Japan). More precisely, the system consisted of a model SCL-10A controller unit, a model DGU-2A degasser unit, an LC-20AD quaternary gradient pump, SIL10AD refrigerated autosampler and a Model SPD-M10AVP PDA detector. System control, data acquisition and processing were performed with a PC-Pentium IV Processor personal computer operated with Microsoft windows 7 and shimadzu LC solution 1.24 SP1 software. Standard substances were weighed on AY 220 shimadzu analytical balance. A glass vacuum filtration apparatus (Alltech Associates) was employed for the filtration of buffer solution using 0. 45 um filter obtained from Pall Life Sciences. Degassing of the mobile phase was performed by sonication in oscar micro clean-103 ultrasonic bath. The drug was successfully eluted on an HIBAR C18 (250 × 4.6 mm i.d., 5µ) column, in 70:30 ratio of acetonitrile and water (mixed with 1%v/v triethylamine; pH adjusted to 4.50±0.05 with 10%v/v orthophosphoric acid) as the mobile phase. The PDA detector set at 227 nm was used to monitor the paclitaxel peak.

DSC studies

Differential scanning calorimetry was performed using DSC-60, shimadzu japan, the instrument comprised of the calorimeter, flow, controller, thermal analyzer and operating software TA 60 from shimadzu corporation japan. The samples were placed in a sealed aluminum pan, before heating under nitrogen flow (30 ml/min) at a scanning rate of 5 °C/min from 25 °C to 250 °C. The heat flow as a function of temperature was measured for the drug and the former crystal mixture.

XRPD studies

This method is based on an interaction of a monochromatic X-ray beam with a crystalline substance. Different planes of atoms or molecules in a crystal act as a grating for X-rays. For monochromatic X-rays, the diffraction angle solely depends on the crystalline spacings. The patterns provided by X-ray diffraction i.e. intensity vs. scattering angles is unique for each crystalline form of the compound. PXRD measurements were conducted using rigaku mini flex 600 X-ray diffractometer (Rigaku Co., Tokyo, Japan). The instrument was operated at 600 watts (X-ray tube), with a fixed tube current of 15 mA and a voltage of 40 kV. The diffracted X-ray beam was monochromated by a graphite monochromator and detected using standard scintillation counter. Diffraction intensities were measured by fixed time step scanning method in the range of 5-400 (2Θ).

FTIR studies

FTIR spectrum was generated for the prepared cocrystals using a Shimadzu FTIR-8300 spectrophotometer, and the spectrum was recorded in the region of 4000 to 400 cm-1. The procedure consisted of dispersing a sample (drug and cocrystals formers) in KBr and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path, and the spectrum was recorded for the all the batches of cocrystals.

RESULTS AND DISCUSSION

Solid state characterization of cocrystals

Before cocrystals can be formulated into medicines, it is essential that their solid-state chemistry and stability is understood. It is also extremely useful to determine the crystal structure of the cocrystals, as this can provide significant new insights into how the drug and conformer interact, and thereby provide an excellent crystal engineering guide to new cocrystals, potentially with improved properties. Single-crystal X-ray diffraction will be used to determine their crystal structures; differential scanning calorimetry, X-ray powder diffraction (including variable-temperature), Raman spectroscopy (including again variable temperature) and hot-stage microscopy will be used to determine their stability and any phase transformations (including decomposition) which they might undergo as a function of temperature.

The results indicate that peaks present in FTIR spectra of the drug are similar to peaks present in FTIR spectra of cocrystals of CCN-1 with a marginal decrease in intensity and broadening of the peak at wave number 2994.00 cm-1 was observed due to the involvement of N-H hydrogen of secondary amide in intermolecular hydrogen bonding. This formation of hydrogen bonding is due to the involvement of N-H hydrogen of secondary amide in intermolecular hydrogen bonding. Thus the results of FTIR spectroscopy support the formation of intermolecular hydrogen bonding which are the characteristics of cocrystals, thus the results of FTIR spectroscopy support the formation of intermolecular hydrogen bonding which are the characteristics of cocrystals. (table no.2 and fig. no.3 and 4).

Table 2: Major IR peaks (wavenumbers) and DSC thermograms results of cocrystals

Batch No.	Samples	Composition	Major peaks (Wave numbers, cm^{-1}) (By FTIR)	Melting points (By DSC)
CCN-1	Paclitaxel: NAR CCN-1	1:1	3416.05(N-H), 2360.95,1643.41(C=0),, 1246.06, 1070.53, 707.90	209.60 ° C

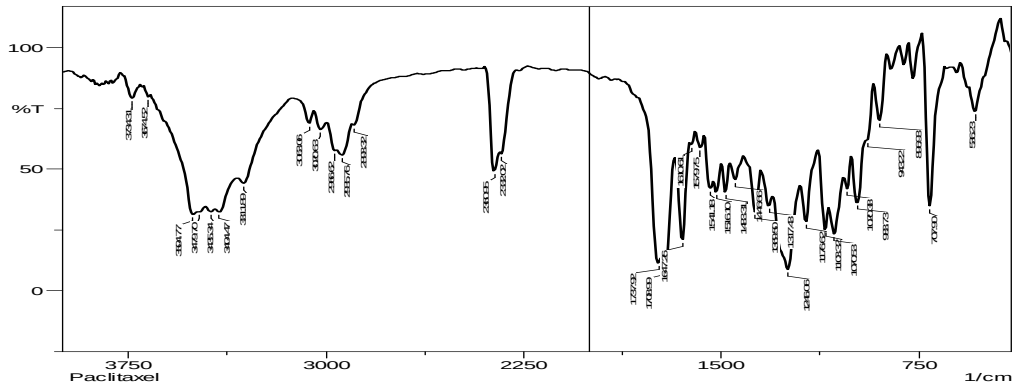

Fig. 3: FTIR spectra of PTX

The DSC thermograms of the pure drug showed a sharp endothermic peak at 223.00 °C. Paclitaxel cocrystals showed sharp endothermic @ 320.00 °C; the results indicate that intensity of endothermic peaks observed in cocrystals was slightly lesser and distinct, with a different melting transition from that observed with either of the individual components, resulting in the formation of new crystalline phase. (table 2: and fig. 5 and 6).

The pure drug and cocrystals are demonstrated by the diffraction patterns. The intensity of X-ray diffraction pattern for the pure drug, at a 2 Θ angle of 12.22 was found to be 100%. Whereas, cocrystals showed 100% intensity at 22.10 2 Θ angle. The shifting of 100% intensity for 2 Θ angle in comparison with pure and cocrystals is mainly because of interplanar distance (d angle) indicating the different arrangement of molecules, hence confirms the development of new crystalline phase. (table 3: and fig. 7 and 8).

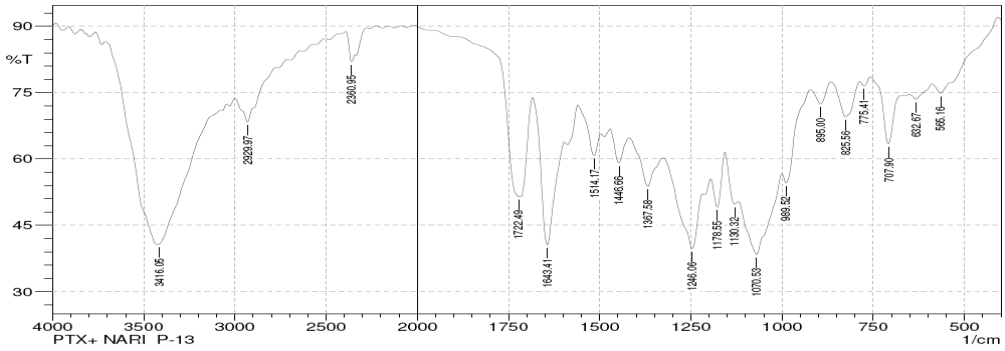

Fig. 4: FTIR spectra of PTX: NAR-CCN-1

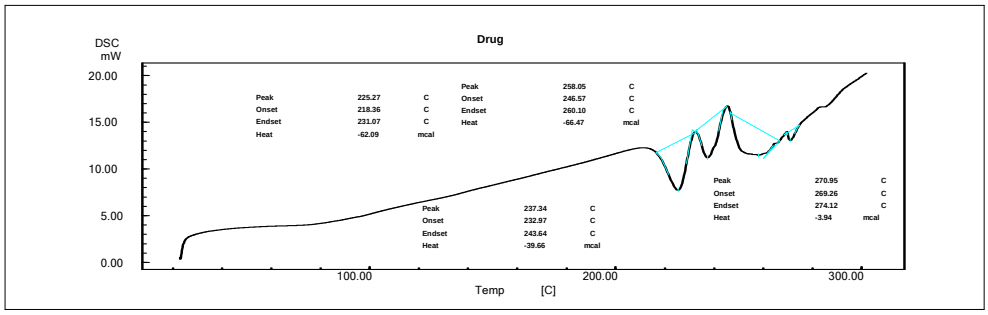

Fig. 5: DSC Thermogram of PTX

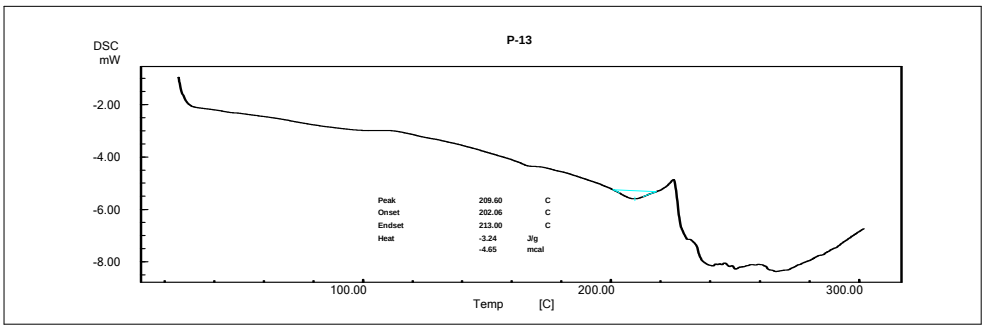

Fig. 6: DSC Thermogram of PTX: NAR-CCN-1

Table 3: PXRD data of cocrystals and paclitaxel

Sample	2θ (deg)	d (ang.)	Intensity
Paclitaxel	12.22	7.23	2487.74
Paclitaxel: NAR(1:1) Co-crystal (CCN-1)	22.10	4.01	3121.08

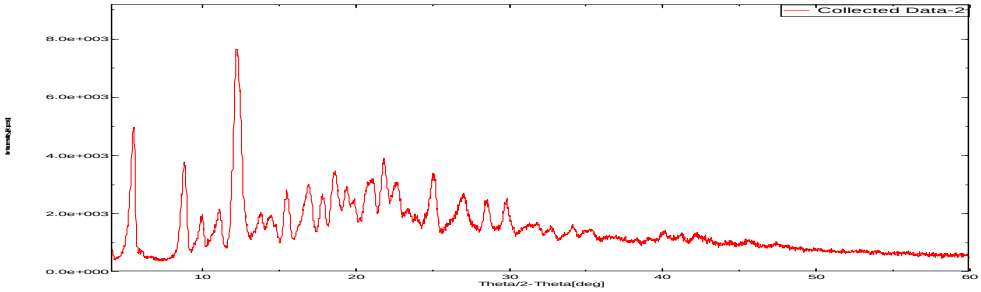

Fig. 7: PXRD of PTX

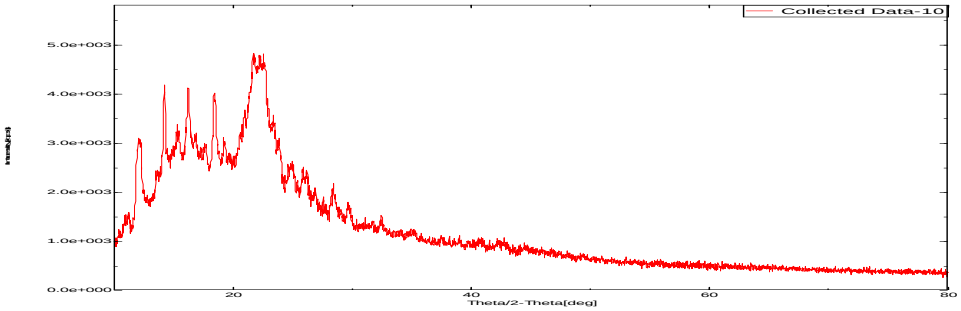

Fig. 8: PXRD of PTX: NAR-CCN-1

Chemical stability

The drug content of the optimized cocrystals CCN-1 was calculated using RP-HPLC technique and was found to be within and 98.86±0.13 also the peak purity results shows that drug is stable, and no degradation peaks were interfering the results and with 0.99988 of total peak purity index. The peak purity index of the paclitaxel was passed the criteria. Paclitaxel eluted at retention time of 4.80±0.032 min and total chromatographic run time was 7.00 min. (fig. no.09).

Physical stability

The optimized CCN-1 was found to be extremely stable after a maximum period of 90 d, at 25 ° and 40 °C which was confirmed by X-ray diffractogram and microscopic methods using stage and eyepiece micrometers to determine the change in the appearance of the cocrystals with respect to initial zero hour analysis.

Solubility studies of prepared cocrystals

From the results, it was observed that the batch CCN-1 shows statistically significant enhancement in the saturation solubilities as compared to their other batches and pure drug indicating the formation of cocrystals. (table 4:)

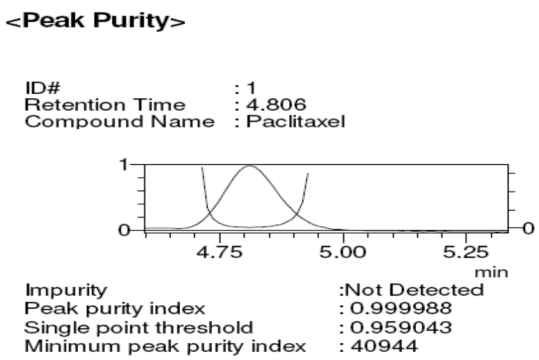

Fig. 9: Peak purity curve for the cocrystals PTX: NAR-CCN-1 for proving chemical stability

Table 4: Saturation solubility studies of cocrystals

Sample (n=3)	Solubility in ultra-clear water in µg ml^{-1}±SD	Solubility In pH 6.8 phosphate buffer in µg ml^{-1}±SD
Paclitaxel	1.34±0.25 µg ml^{-1}	1.38±0.41 µg ml^{-1}
Paclitaxel: NAR(1:1) Co-crystal (CCN-1)	3.17±0.87 µg ml^{-1}	3.22±0.65 µg ml^{-1}
Paclitaxel: NAR(1:2) Co-crystal (CCN-1)	2.14±0.28 µg ml^{-1}	2.29±0.73 µg ml^{-1}
Paclitaxel: NAR(2:1) Co-crystal (CCN-1)	1.64±0.36 µg ml^{-1}	1.76±0.12 µg ml^{-1}

CONCLUSION

Paclitaxel cocrystals were prepared by solvent-assisted co-grinding method with the aim of increasing the solubility of the drug. Batches were made using naringen in different molar ratios.

The most important conclusions of the present work can be summarized as:

Paclitaxel was able to form stable cocrystals with Naringen in different ratios. The cocrystals formed were characterized and the analysis for saturation solubility and dissolution was carried out. Cocrystals of Paclitaxel: NAR in 1:1, 1:2 and 2:1 molar ratios were prepared by solvent-assisted co-grinding method. The formation of the cocrystals was confirmed by solid-state characterization of Paclitaxel: NAR(1:1) Co-crystal (CCN-1) batch based on the solubility studies which included PXRD, DSC and FTIR, the results of which proved the structural modifications in the cocrystal. PXRD results exhibited changes in the peak locations and patterns indicating the development of new crystalline phase. DSC thermogram showed the endothermic peaks at 223.00 °C for pure PTX, whereas endothermic peaks for cocrystals were observed at 320.00 °C for cocrystals which support the above findings. These results were also supported by FTIR spectrum that confirmed the hydrogen bond formation. The cocrystals were found to be physically and chemically stable. A 2.4 fold increase in the saturation solubility of Paclitaxel: NAR(1:1) Co-crystal (CCN-1) was observed in its co-crystallized form.

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CONFLICT OF INTERESTS

Declare none.

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