

Experimental Data of Fabricated Co-crystals of Doxorubicin HCl with Flavonoids

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ABSTRACT

Objectives: This current research work aimed for improvement of the solubility and permeability of poorly permeable Doxorubicin HCl through generating co-crystals. The main goal for the preparation of oral drugs is to decrease the overall cost of healthcare and route of administration is made easy. **Methodology:** In this study co-crystals of Doxorubicin HCl with Quercetin hydrate and Naringin has been prepared based on ease of hydrogen bond formation. The co-crystal batches of Doxorubicin HCl-Quercetin hydrate in ratio 1:1, 1:2, 2:1 and Doxorubicin HCl-Naringin in ratio 1:1 are prepared by slow solvent evaporation technique. **Results and Discussion:** The formation of co-crystal was confirmed by PXRD, DSC and FTIR. Doxorubicin HCl with Naringin crystal batch show improved solubility. The dynamic solubility of Doxorubicin HCl-Naringin co-crystal in the ratios 1:1 increased by approximately 9.2-fold as compared to pure drug and Doxorubicin HCl -Quercetin hydrate cocrystal batches.

Key words: Doxorubicin HCl, Quercetin hydrate, Naringin, Cocrystals, HPLC, Solid state characterisation, Saturated solubility.

INTRODUCTION

In the pharmaceutical industry, only 1% of pharmaceutically active compounds are commercialised because of its poor biopharmaceutical properties when compared to toxicity or lack of efficacy. The solubility remains the key issue among these biopharmaceutical properties as opposed to lethality or absence of adequacy. The solubility remains the key issue among these biopharmaceutical properties. As indicated by the investigations, 75% of the medication advancement had low solubility. Various methods are introduced to seek out this issue such as micronization, the usage of salt forms and solubilisation in solvents and micelle arrangements. Even though these techniques can be effective, there is a requirement for a for a broader range of solid forms from which to pick a specific API in order to upgrade physicochemical properties without altering the atom itself.¹⁻³

Advances in crystal engineering have allowed the design of cocrystals in which two or more compounds in specific stoichiometric ratio are incorporated into the same crystalline lattices.^{1,4}

This mainly helps us to increase the solid-state bioavailability of the drug, thus, oral treatment with anti cancer agents is feasible to be preferred, as oral administration is convenient for patients and reduces administration costs. The main goal for the preparation of oral drugs is to decrease the overall cost of healthcare and route of administration is made easy.⁵ Doxorubicin hydrochloride (DOX) belongs to BCS class III drug. It is an antibiotic cytotoxic anthracycline isolated from organism cultures *Streptomyces peucetius* var. *caesius*. The most common uses of DOX in malignancy treatment for various types of testicular malignancy,

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leukemia, Ewing's sarcoma, Hodgkin's disease and Kaposi's sarcoma.⁶

DOX is chemically 5, 12-Naphthacenedione,10-[(3-amino-2,3,6-trideoxy- α -L-xylo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy, hydrochloride. Its empirical formula is $C_{27}H_{29}NO_{11} \cdot HCl$ and molecular weight is 543.52g/mol.⁷ Doxorubicin HCl is a cytotoxic anthracycline antibiotic and like all anthracyclines, it works by intercalating DNA with the most serious adverse effect being life-threatening heart damage. It is prescribed in the treatment of a wide variety of malignant neoplastic diseases.⁶ It is only approved for intravenous administration (infusion) for 3 to 10 min, but the main drawback is it may increase the risk of CHF. It cannot be administered orally due to its low bioavailability (approx. < 5%) resulting of poor permeability. It exhibits low oral bioavailability due to active efflux from intestinal P-glycoprotein receptors⁸ so by the help of crystallization there may a chance for binding to the p-glycoprotein receptors due to change in structure of the drug. Clearly, there is an opportunity to create oral dosage forms of drugs with poor permeability. This current research work aimed for improvement of the solubility and permeability of poorly permeable Doxorubicin HCl through generating co-crystals.

Naringin is one of the widely available natural flavonoids; it inhibits cytochrome enzymes. Using naringin as conformer we are going to develop doxorubicin crystals with enhanced solubility while maintaining or improving other relevant physical properties (stability, process ability, etc.).

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) belongs to a subclass of flavonoids called flavanols. Quercetin exists as yellow needles or a yellow powder. It is used in the treatment of cancer and a number of other diseases. It is also known to act as an antiplatelet agent and improve the overall function of the heart hence used as a co-former in the present research.⁹

MATERIALS AND METHODS

Materials

Doxorubicin HCl (DOX); Quercetin Hydrate (QUE), Naringin (NAR) was purchased from Sigma-Aldrich. Analytical grade methanol was obtained from FINAR Limited and acetone from Merck Life Sciences Private Limited. A Water purification system (Siemens AG, Germany) was used in the laboratory to obtain HPLC grade water.

Methods

Selection of coformer

Generally, a coformer will be selected based on trial and error but this approach is void and time consuming. Other approaches available are Cambridge structural database and supramolecular synthon approach. In this research supramolecular symphony approach was used to select the coformer which shows us the ease of hydrogen bonding between functional groups present in the API and the conformer.¹⁰ Crystal solubility increases effectively by selecting a coformer with higher solubility, hence selection of coformer plays a crucial role.

Quercetin hydrate and Naringin was selected as co-former after promising results in hydrogen bonding.^{2,9} Doxorubicin HCl has 6 hydrogen bond donor sites and 12 hydrogen bond acceptor sites resulting in a high probability of co-crystal formation with cofomers.¹¹

Co-crystal preparation

Co-crystals were prepared in stoichiometric ratios 1:1, 1:2, 2:1 of Doxorubicin-Quercetin hydrate and 1:1 stoichiometric ratio of Doxorubicin-Naringin by slow solvent evaporation method. Table 1(A), 1(B) shows mass needed for the preparation of cocrystals. Required amount of co-crystal component was weighed and solvent is added slowly to prepare saturated solutions of each component separately. Acetone: methanol (50:50 % v/v) is used as a solvent. The saturated solutions were mixed in a beaker and vortexed for 10 min. The vortexed solution is poured into a petri dish and spread evenly and covered with aluminium foil with holes. The solution is allowed to evaporate at room temperature until the

Table 1: (A) Mass needed for preparation of Doxorubicin HCL-Quercetin hydrate co-crystals in specific ratios.

Doxorubicin: Quercetin hydrate ratio	Doxorubicin [mg]	Quercetin hydrate [mg]	Batch size
1:1	157.310	92.689	250mg
1:2	229.523	270.476	500mg
2:1	386.217	113.782	500mg
1:1 physical mix.	157.313	92.686	250mg

Table 1: (B) Mass needed for preparation of Doxorubicin HCL- Naringin co-crystals in specific ratios.

Doxorubicin HCl: Naringin Ratio	Doxorubicin HCl (mg)	Naringin (mg)	Batch size
1:1	241.767	258.232	500mg
1:1 physical mix.	241.767	258.232	500mg

sample is completely dry. The petri dish was scraped and dried the co-crystals were collected and stored for solid state analysis.

The 1:1 molar ratios of physical mixture samples were also prepared.

Analysis

DSC

Shimadzu TA-60WS thermal analyzer was used to obtain DSC analysis. 5mg of sample were placed in an aluminum pans (0.1mm thickness) and then crimped with an aluminum lid. Then the samples are placed into sample holder and allowed to heat from the range 30°C to 350°C at a fixed heating rate (10°C per min) under a nitrogen flow (10cc/min). Powdered alumina (5mg, 100mesh) is used as a reference for the calibration of heat flow and heat capacity signals.

FTIR

Shimadzu FTIR-8300 system (Kyoto, Japan) was used to obtain the spectra of the prepared co crystals. The spectrum was collected over a range of 4000-500cm⁻¹. (25 scans, resolution 4cm⁻¹). Preparation of the disc involved dispersing the sample in Kerr and then grinding with applied pressure (1000psig).

HPLC Method

The co-crystal purity was analyzed with a Shimadzu LC-10 series chromatographic system. The system as a controller unit (SCL-10A VP), a degasser unit (DGU-20A5), a quaternary gradient pump (LC-20AD), a refrigerated auto sampler (SIL-20AC HT) and a PDA Detector (SPD20A). The buffer solution was filtered with a 0.45µm filter (Pall Life Sciences) vacuum filtration device. In the Equitron ultrasonic bath, the mobile phase was degassed by sonication. For the stationary phase a Phenomena C₁₈ (150 × 4.6mm × 5) column was used and the mobile phase was a mixture of acetonitrile: 0.1% phosphoric acid in water (40:60 % v/v). The flow rate was 0.75mL/min and detection wavelength at 254 nm at 35°C.

XRD

Rigakuminiflex 600 X-ray diffract meter (Riau 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 X-ray beam (diffracted) was monochromatic by a graphite monochromatic and detection was carried out by a standard scintillation counter. Diffraction intensities were measured over a range of 5–80°(2θ).

Saturated solubility studies

Saturation solubility studies Shake flask method was used to determine the equilibrium dynamic solubility on

prepared crystal batches of Doxorubicin HCl-Quercetin hydrate [(1:1), (1:2), (2:1)], Doxorubicin HCl-Naringin [1:1] and physical mixtures. IP phosphate buffer (pH 6.8) was used. IP phosphate buffer (pH 6.8) was used since it mimics the conditions of intestine pH. Samples were prepared by adding excess quantities of each into injection vials containing 2mL of the solvents. Thereafter all the samples were shaken in Laptop orbital shaking incubator maintaining the temperature 37°C at 150rpm. Samples were collected after 24 hr 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 the developed HPLC method. The samples were quantified using developed HPLC method with PDA detector.

RESULTS AND DISCUSSION

Solid State Characterization

XRD

The interaction between the drug and the conformer is indicated by the difference in 2θ values of pure drug and crystals. Very slight change in the diffraction patterns were seen. The pure drug Figure 1(A) showed sharp peaks whereas the crystals Figure 1(B) did not show sharp peaks which suggests that there is interaction. Though the diffraction of crystals differs from that of pure drug, we cannot conclude that a new crystalline structure is formed because of slight amorphous halo nature of the crystal diffraction pattern. Table 2 shows the intensity and 2θ degree values of crystal batch and drug.

FTIR

(A)The FTIR spectra of DOX-QUE 1:1 [Figure 2(B)] batch revealed that crystal were formed as spectra was similar with less intensity and broadening at 3024cm⁻¹ and the Cl group present in the Figure 2(A) show spectra at 999cm⁻¹ with more intensity but there are no spectra at 999cm⁻¹ in crystal spectra Figure 2(B). The spectra at 1730cm⁻¹ is also changed in crystal when compared to Figure 2A.

(B)The FTIR spectra of DOX-QUE 1:2 [Figure 2(C)] batch revealed that crystal were formed. As spectra was similar to peaks present in FTIR spectra of Figure 2(A) with a marginal decrease in intensity and broadening of the peak at 3280 cm⁻¹ Figure 2(C).

Table 2: XRD results.

Sample id	2θdegree	d(angle)	Intensity
Doxorubicin HCL	22.125	4.01	1152.17
Doxorubicin: Quercetin hydrate (1:1)	22.315	3.98	6642.63

(C)The FTIR spectra of DOX-QUE 2:1[Figure 2(D)] batch revealed that crystal were formed as spectra was different for pure and crystal. In Figure 2(D) we find spectra at 2358.94 with sharp peak which shows strong N=H and broadening of spectra at 3329cm⁻¹ shows OH interactions which are different from Figure 2(A).

(D)The FTIR spectra of DOX-NAR 1:1[Figure 2(E)] batch revealed that crystal were formed as spectra has broadening at 3024cm due to hydrogen bonding.

DSC

The Figure 3(A) and Figure 3(B) shows the thermograms of the pure drug, pure conformers and the prepared crystal batches. The endothermic peaks of the crystal batches are different when compared to the pure drug and conformers which indicated formation of crystals. Table 3 shows the melting endotherm values of pure drug and prepared crystal batches.

Saturation Solubility

Solubility studies are done and samples and taken after 24 hr for analyzing the solubility of the pure drug and

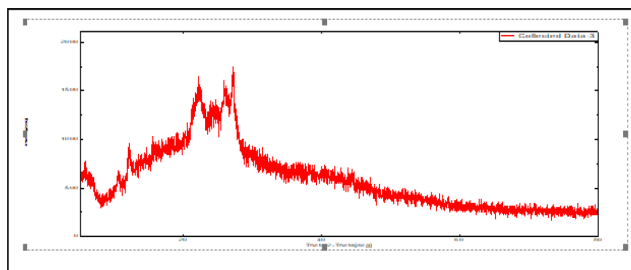


Figure 1(A): XRD of Pure drug.

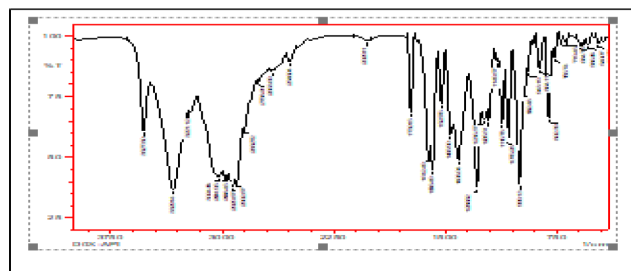


Figure 1: (B) XRD of Doxorubicin-Quercetin hydrate cocrystal (1:1)

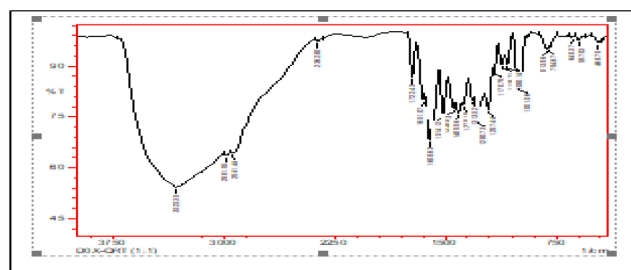


Figure 2(B): FT-IR spectra of DOX-QUE 1:1 batch.

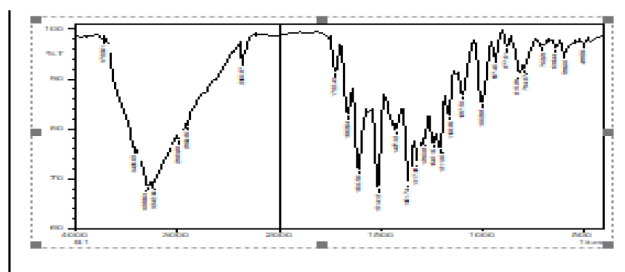


Figure 2(C): FT-IR spectra of DOX-QUE 1:2 batch.

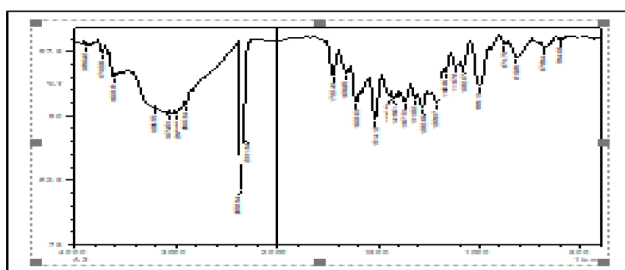


Figure 2(D): FT-IR spectra of DOX-QUE 2:1 batch.

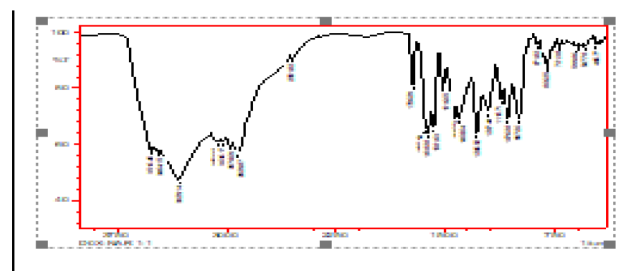


Figure 2: (E) FT-IR spectra of DOX-NAR 1:1 batch.

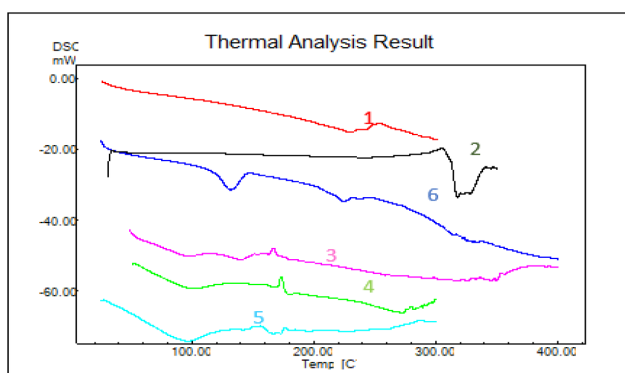


Figure 3(A): DSC thermogram of DOX-QUE batches [1) Pure DOX, 2) Pure Quercetin hydrate, 3) DOX-QUE [1:1], 4) DOX-QUE [1:2], 5) DOX-QUE [2:1], 6) DOX-QUE physical mixture].

prepared crystals batches. There were analyzed by using optimized HPLC. Table 4 shows the saturation solubility data.

As shown in Figure 4, Doxorubicin HCL-Naringin co-crystal (1:1) form shows approximately 9.2-fold increase in solubility as compared to its pure form,

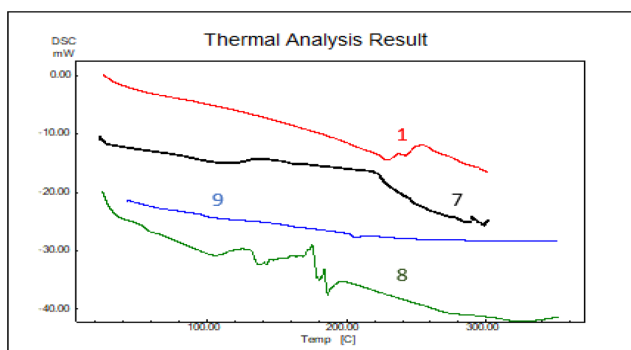


Figure 3(B): DSC thermogram of DOX-NAR batches [7] Pure Naringin, 8) DOX- NAR 1:1, 9) DOX- NAR physical mixture].

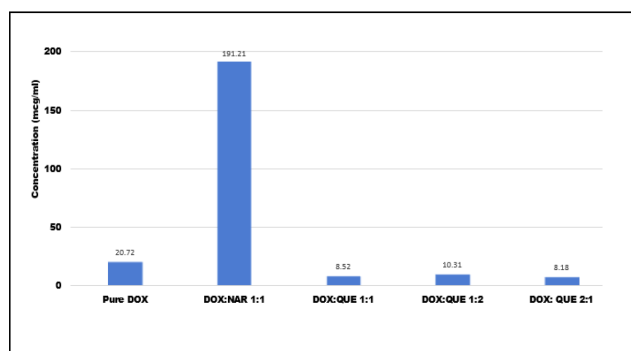


Figure 4: Saturation solubility studies of batches of Cocryystals, pure drug and in phosphate buffer pH 6.8.

Table 3: DSC thermogram melting point values for pure drug, pure conformers and crystal (CC) batches.

Sample	Endotherms point [°C]
Doxorubicin HCL	216
Quercetin hydrate	300
Naringin	160
1:1DOX-QUE CC	131.9;224.82
1:2 DOX-QUE CC	98.0;139.68
2:1 DOX-QUE CC	101.09;271.9
1:1 DOX-NAR CC	186.5
1:1 DOX-QUE physical mix	131.76;158.93
1:1 DOX-NAR physical mix	206.53

physical mixture and crystal batches of DOX-QUE batches.

CONCLUSION

Data in this article shows the characteristics of products prepared from different molar ratios of Doxorubicin HCl- Quercetin hydrate and Doxorubicin HCl -Naringin. Table 3 shows the melting

Table 4: Saturation solubility data.

Batch	Solubility in phosphate buffer (pH6.8 ,37°C)
Pure Drug	20.72 µg/ml
DOX-NAR(1:1) CC	191.21 µg/ml
DOX-QUE(1:1) CC	8.52 µg/ml
DOX-QUE(1:2) CC	10.31 µg/ml
DOX-QUE(2:1) CC	8.18 µg/ml

Points of pure reactants and of the product samples and Figure 3 displays the complementary thermo grams. Figure 2 displays the FTIR spectra of the samples. All four sample batches prepared by slow evaporation method show a broadening (3329-2900 cm^{-1}) may be due to hydrogen bonding. Table 2 and Figure 1A, 1B displays XRD results. The pure drug showed sharp peaks whereas the cocryystals did not show sharp peaks which suggests that there is interaction. Table 4 and Figure 4 shows saturated solubility studies. Doxorubicin-Naringen co-crystal (1:1) form shows approximately 9.2-fold increase in solubility as compared to its pure Doxorubicin and Doxorubicin-Quercetin hydrate crystal. Finally, it could be concluded that the co-crystals of Doxorubicin HCl with Naringen by slow evaporation technique improved the solubility. Therefore, co-crystal formation by slow evaporation technique might be a useful strategy for increasing the therapeutic potential of Doxorubicin HCl.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

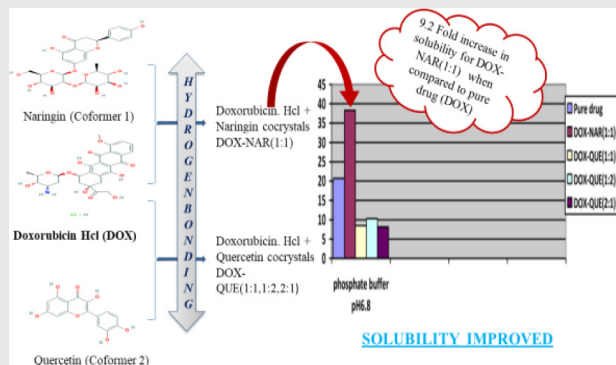
DOX: Doxorubicin HCl; **QUE:** Quercetin hydrate; **NAR:** Naringin; **CC:** Cocryystal; **HPLC:** High Performance Liquid Chromatography; **IP:** Indian Pharmacopoeia.

REFERENCES

- Aakeröy CB, Forbes S, Desper J. Using crystals to systematically modulate aqueous solubility and melting behavior of an anticancer drug. *Journal of the American Chemical Society*. 2009;131(47):17048-9.
- Schultheiss N, Newman A. *Pharmaceutical Crystals and Their Physicochemical Properties*. *Crystal Growth and Design*. 2009;9(6):2950-67.
- Mirza S, Miroshnyk I, Heinämäki J, Yliruusi J. Co-crystals: An emerging approach for enhancing properties of pharmaceutical solids. *Dosis*. 2008;24(2):90-6.

- Jasud S, Warad S, Rahul S, Jagdale G, Zinjad S. Crystal: A Novel Approach for Bioavailability Enhancement. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2013;2(6):4682-97.
- Muddukrishna BS, Swapnil JD, Gautham GS, Krishnamurthy B. Preparation, Solid State Characterisation of Paclitaxel and NaNaringen co-crystals with Improved Solubility. *International Journal of Applied Pharmaceutics*. 2016;8(4):32-7.
- Pradhan N, Rajkhowa H, Giri H, Shrestha B. Simultaneous Spectrophotometric Estimation of Moxifloxacin Hydrochloride and Doxorubicin Hydrochloride. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2015;7(11):21-6.
- Manasa E, Prakash KV, Pratap PR, Susena S. Method Development and Validation of Doxorubicin Hcl In Api And Its Formulation By Spectrophotometry. *International Journal of Pharmaceutical: Chemical and Biological Sciences*. 2013;3(4).
- Hetal T, Bindesh P, Sneha T. A review on techniques for oral bioavailability enhancement of drugs. *Health*. 2010;4(3):203-23.
- Nijveldt RJ, Nood ELV, Hoom DEV, Boelens PG, Norren KV, Leeuwen PAV. Flavonoids: A review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*. 2001;74(4):418-25.
- Wouters J, Quéré L. *Pharmaceutical salts and co-crystals*. Royal Society of Chemistry. 2012.
- Pubchem, <https://pubchem.ncbi.nlm.nih.gov>.

PICTORIAL ABSTRACT



SUMMARY

CoCrystals of Doxorubicin HCl with Quercetin hydrate and Naringen was prepared by slow solvent evaporation. Solid state characterization studies (DSC, FTIR, XRD) are done for the prepared crystal batches which shows the different characterization for pure Drug and prepared crystal batches. Hence it says there is an interaction between the Pure drug and the conformers. The saturated solubility studies data shows 9.2-fold increase in the solubility of Doxorubicin-Naringen co-crystal (1:1) as compared to its pure Doxorubicin and Doxorubicin-Quercetin hydrate crystal batches.

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