

# Enhancement of Solubility of Antifungal Ketoconazole by Co-crystallization

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## ABSTRACT

Ketoconazole is available in various oral and topical forms. It is a weak base and has limited solubility except in highly acidic conditions. It falls under Class II in the Biopharmaceutics Classification Scheme due to high permeability but low water solubility (0.087 mg/l at 25 °C). A cocrystal of ketoconazole was synthesized using the solvent crystallization method and characterized using FTIR spectroscopy, DSC, melting point, and solubility analysis. The FTIR spectrum indicated non-covalent interactions (hydrogen bonding) in the cocrystal, resulting in improved physical properties compared to the individual components. The melting point of the cocrystal was lower than that of the single API (146.0-148.0 °C), specifically (141.0-143.0 °C) and (115.0-119.0 °C) for suberic acid and sebacic acid cocrystals, respectively. The ketoconazole cocrystal exhibited enhanced solubility compared to pure ketoconazole. This study demonstrated the successful formation of co-crystals between ketoconazole and suberic acid, sebacic acid, and various co-solvents, leading to improved drug solubility.

**Keywords:** Cocrystal, non covalent interactions, enhanced solubility, and improved physical properties.

## 1. INTRODUCTION

Solubility is a crucial property in the field of drug discovery and development, as it affects various stages of the process. It is particularly significant in target identification, lead optimization, preclinical and clinical trials. Solubility also plays a vital role in the design and development of different dosage forms for various routes of administration, including oral, dermal, parenteral, and pulmonary. Drugs with poor water solubility often exhibit limited and inconsistent bioavailability. The low solubility and dissolution rate of such drugs can greatly impact their therapeutic efficacy and significantly reduce their market value. To address this issue, multi-component crystals, such as solvates, hydrates, co-crystals, and salts, have emerged as important tools to enhance solubility (Sekhon et al., 2009).

Pharmaceutical co-crystals are unique crystalline solids that consist of two neutral molecules. One molecule is the active pharmaceutical ingredient (API), while the other is a co-crystal former. The co-crystal former can be an excipient or another drug. These co-crystal components are solid at room temperature and exist in a specific stoichiometric ratio. They interact through noncovalent interactions, predominantly hydrogen bonds, which define and create new formulations of drugs. Pharmaceutical co-

crystal formation has shown promise in improving the performance of drugs with poor solubility (Jayasanker et al., 2006) Overall, pharmaceutical co-crystals offer a valuable opportunity to enhance the solubility and formulation options of APIs. They have the potential to address the challenges associated with poor solubility, ultimately improving the effectiveness and market value of drugs in the pharmaceutical industry. Cocrystals have been developed to enhance the properties of active pharmaceutical ingredients (APIs), such as solubility, chemical stability, and bioavailability (Rodriquez-Hornedo et al., 2007). Various methods have been used for co-crystal synthesis, including solvent evaporation, slurry conversion, grinding, melting, sublimation, and electrospray. However, these techniques often result in the crystallization of individual components which require complex manufacturing processes, specialized equipment, large amounts of raw materials, and may involve regulatory complications and time-consuming steps (Indra et al., 2019).The solvent crystallization method is employed to achieve super-saturation beyond the concentration ratios of co-crystal components in the solution. Cofomers, which are like pharmaceutical excipients, are an integral part of pharmaceutical cocrystals. They form hydrogen bonds with the API, providing stability to the co-crystal.

Cofomers should be non-toxic, devoid of adverse effects, and included on regulatory lists such as the USFDA and EAFUS (Remenar et al., 2003).

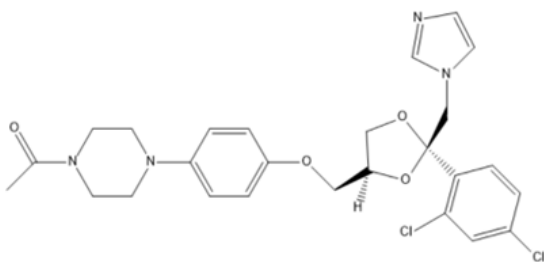


Figure 1: Molecular structure of KTZ

The selection of cofomers is based on their ability to form complementary hydrogen bonds with the API. Hydrogen bonds play a crucial role in molecular recognition due to their direct interactions. One challenge in producing cocrystal pharmaceuticals is selecting cofomers that are compatible with a specific Active Pharmaceutical Ingredient (API) (Kamble et al., 2017). Carboxylic acids are strong electron acceptors and donors, and they play a significant role in supramolecular reactions. Carboxylic acids are generally recognized as safe (GRAS) by the FDA and can be used as cocrystal formers in combination with an API. A common approach to cofomer selection is through “tactless” cocrystal screening. This method involves using a predetermined library of pharmaceutically acceptable/approved compounds to attempt co crystallization (Korotkova et al., 2014).

Regarding the specific API mentioned, ketoconazole (cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine), Fig. 1, it would require a compatible cofomer selection for cocrystallization. Ketoconazole (KTZ) is a broad-spectrum imidazole antifungal agent that can be administered topically or orally. It belongs to Biopharmaceutics Classification Scheme (BCS) Class II drugs, which are characterized by high permeability but low solubility in water. The chemical structure of ketoconazole has a visible hydrophobic site and weak basicity, which contribute to its poor aqueous solubility. Commercially, ketoconazole is available in various dosage forms for oral and topical administration. However, it has low and variable bioavailability, and there is a risk of hepatotoxicity associated with its oral use. Due to these concerns, the therapeutic indications for oral ketoconazole have been restricted, and there has been a need to re-evaluate its risk/benefit profile (Fukte et al., 2014). Increasing the bioavailability of ketoconazole would allow for lower therapeutic doses, reducing the risk of hepatotoxicity.

Ketoconazole is practically insoluble in water

but freely soluble in dichloromethane, soluble in methanol, and sparingly soluble in ethanol (Choi et al., 2008). In this work, the formation of cocrystals of ketoconazole with acceptable pharmaceutical cofomers, namely suberic acid and sebacic acid, was evaluated. Cocrystals are formed by combining two or more components in a crystal lattice structure, aiming to improve the physicochemical properties of the active pharmaceutical ingredient (API) (Aggarwal et al., 2011). Overall, the information provided highlights the solubility challenges of ketoconazole, the evaluation of cocrystals as a potential solution, for the delivery system for pharmaceutical compounds (Etter et al, 1991).

## 2. METHODS AND EXPERIMENTAL

### 2.1. MATERIALS

Ketoconazole (KTZ) (Al-Quds Pharmaceutical Company, Piramal Enterprises Limited), Methanol 99% (Sigma Aldrich), Acetone 99% (Chemistry Lab at Al-Quds University), Suberic acid 98% (Mw: 174.19, mp: 139-146 °C), Sebacic acid 99% (Mw: 202.25, mp: 133-137 °C (lit.) (Sigma Aldrich), , Double distilled water (Chemical Analysis Laboratory at al-Quds University), Ethanol. All chemicals were used as supplied and tested at al-Quds University.

### 2.2. EXPERIMENTAL

Ketoconazole was used as the active pharmaceutical ingredient (API) in this study. The co-crystal technique of solvent crystallization was employed, utilizing methanol (99%) as the solvent and a co-solvent. The process involved weighing the diacid and the API in molar ratios (1:1) and grinding them together using a mortar and pestle. The resulting mixture was dissolved in a minimal amount of solvent (a 1:1 mixture of methanol and acetonitrile). The cocrystals of ketoconazole and the dicarboxylic acid cofomers were formed through solvent crystallization at room temperature.

The obtained crystals were separated by filtration using a 0.45 µm membrane and then dried at room temperature for 24 hours. The dried crystals were stored in an airtight container in a desiccated environment. Individual samples of the crystals were collected for testing with FTIR spectroscopy. The spectra of the crystals were compared with those of the pure diacids and the API, which were tested prior to the experiment. Other tests conducted included determining the melting point, assessing solubility in water, and performing differential scanning calorimetry (DSC) analysis.

### 3. RESULTS AND DISCUSSION

The diacids (sebacic acid, suberic acid) combined with KTZ (ketoconazole) using methanol and acetone as solvents successfully produced white co-crystals. The presence of hydroxyl groups in these diacids led to fluctuation and differentiation in hydrogen bonding, resulting in stable co-crystals with the API (Active Pharmaceutical Ingredient).

**Co-crystal Solubility in Water:** Improving the solubility of ketoconazole in pharmaceutical drugs is a crucial objective in co-crystallization. The solubility of each co-crystal was tested by adding approximately 10 mg of each co-crystal to a large test tube, followed by dropwise addition of purified water with stirring.

The volume of water required to dissolve all the co-crystal solids was recorded. The solubility of suberic acid-KTZ co-crystal was found to be 10 mg/22 mL, while the solubility of sebacic acid-KTZ co-crystal was 10 mg/24 mL.

The melting point is a physical property that indicates the temperature at which a substance transitions from the solid phase to the liquid phase at equilibrium. Ketoconazole is a pharmaceutical Active Pharmaceutical Ingredient (API) with a melting point range of 146-148°C. When ketoconazole forms co-crystals with diacids, the resulting co-crystals will have different melting points compared to both the API and the diacids. The specific melting points of the diacids and the co-crystals are listed in Table 1.

Table 1: Melting point results

Material name	Melting point (°C)
ketoconazole (API)	146.0 - 148.0
Suberic acid (diacid)	143.0 - 146.0
Sebacic acid (diacid)	135.0 - 138.0
Suberic acid- ketoconazole co-crystal	141.0 - 143.0
Sebacic acid- ketoconazole co-crystal	115.0 - 119.0

Different melting points of a cocrystal compared to its individual components, ketoconazole and co-former, indicate the formation of the cocrystal. The observed change in melting point supports the successful formation of the cocrystal.

The FTIR spectroscopy analysis of the co-crystal can provide valuable information about the formation and structural changes in the molecules involved. By comparing the FTIR spectra of the individual components (ketoconazole and diacids) with that of

the co-crystal, several changes were observed. First, there was a shift in the position of the FTIR bands corresponding to the functional groups involved in the formation of hydrogen bonds. This shift occurs due to the new arrangement of molecules in the co-crystal, which alters the bonding interactions. The shift in the FTIR bands indicates that the hydrogen bonding network has been modified. Additionally, there may be changes in the shape or intensity of certain peaks in the FTIR spectrum. This variation can be attributed to the formation of the co-crystal, suggesting that a new molecular arrangement has been established. These changes can be indicative of the presence of non-covalent interactions, such as hydrogen bonding, which are characteristic of co-crystals.

FTIR KTZ wavenumber (cm<sup>-1</sup>), 2965, 2883, 1645, 1584, 1555, 1510, 1457, 1415, 1371, 1290, 1243, 1222, 1200, 1174, 1157, 1106, 1079, 1051, 1031, 1005, 981, 967, 905, 864, 839, 813, 791, 736, 718, 703, 673, 663, 665, 627, 587, 566, 510.

FTIR Suberic acid wavenumber (cm<sup>-1</sup>), 2934, 2868, 1685, 1465, 1423, 1408, 1330, 1274, 1249, 1188, 1012, 923, 796, 724, 682, 522.

FTIR suberic acid -KTZ cocrystal wavenumber (cm<sup>-1</sup>), 3853, 3735, 3675, 3124, 2925, 2360, 1707, 1606, 1508, 1466, 1442, 1375, 1326, 1282, 1270, 1243, 1183, 1100, 1082, 1053, 1039, 1017, 987, 972, 930, 909, 870, 817, 788, 753, 728, 708, 675, 659, 595, 566, 546.

There is a clear shift in the peaks (cm<sup>-1</sup>) of free KTZ from 1584, 1510, 1457 towards 1606, 1508, 1442 in the co-crystal. And a shift (cm<sup>-1</sup>), of the free suberic acid 1685, 1465 towards 1707, 1465 in the co-crystal.

FTIR sebacic acid wavenumber (cm<sup>-1</sup>), 2916, 2850, 1687, 1465, 1427, 1408, 1351, 1325, 1298, 1237, 1187, 1048, 923, 754, 724, 677, 550.

FTIR sebacic acid -KTZ cocrystal wavenumber (cm<sup>-1</sup>), 3675, 2922, 2360, 1713, 1646, 1608, 1509, 1456, 1435, 1376, 1342, 1283, 1239, 1223, 1176, 1103, 1080, 1052, 1005, 977, 942, 927, 894, 858, 836, 823, 787, 755, 726, 707, 660, 630, 595, 563, 542.

There is a clear shift in the peaks (cm<sup>-1</sup>) of free KTZ from 1645, 1584, 1510, 1557 towards 1646, 1608, 1509, 1456 in the co-crystal. And a shift (cm<sup>-1</sup>), of the free sebacic acid 1687, 1427 towards 1713, 1427 in the co-crystal.

It's important to note that FTIR spectroscopy alone cannot confirm the crystal structure of the co-crystal. To provide further evidence and confirm the results obtained from FTIR analysis, other characterization techniques can be employed, include X-ray crystallography of single crystals, which can directly determine the crystal structure. Crystals obtained were not suitable for X-ray analysis.

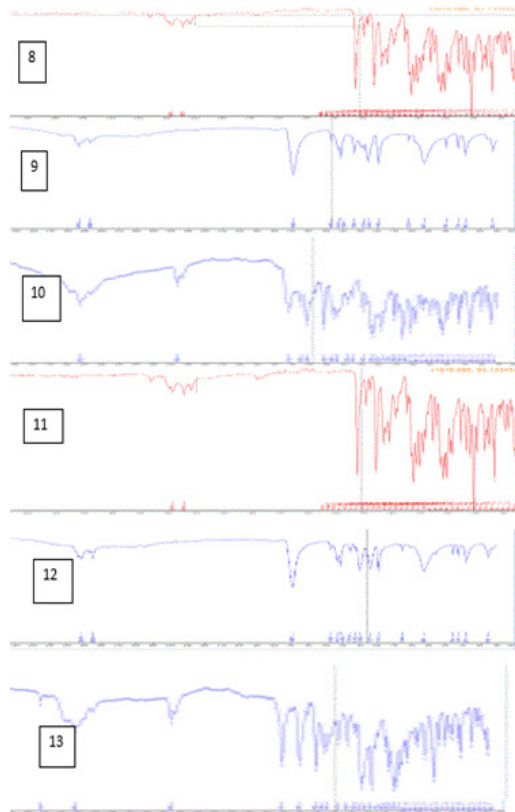


Figure 2: (8) FTIR of KTZ, (9) FTIR of Suberic acid, (10) FTIR of Suberic acid-KTZ cocrystal, (11) FTIR of KTZ, (12) FTIR Sebacic acid, (13) FTIR of Sebacic acid-KTZ cocrystal

DSC study was conducted on pure sebacic acid, suberic acid, ketoconazole, and the co-crystals sebacic acid-ketoconazole and suberic acid-ketoconazole. The results indicate that for sebacic acid, ketoconazole, and the co-crystal sebacic acid-ketoconazole, a sharp exothermic peak was observed, indicating the melting point. The value of  $\Delta H_{fuss}$  could be easily calculated.

The sharpness of the peak suggests the formation of the co-crystal, and the melting occurred at that point, indicating its purity. The melting point of the co-crystal was lower than that of both pure compounds, supporting the formation of the co-crystal. This evidence aligns with previous findings from FTIR, melting point, and solubility analyses.

For suberic acid, ketoconazole, and the co-crystal suberic acid-ketoconazole, the DSC analysis revealed a sharp peak at 143°C, indicating the melting of the pure material. No range in melting temperature was observed. Comparing the melting point of the co-crystal with that of the pure material, it was lower as

expected in cases of co-crystals (Martin 2013).

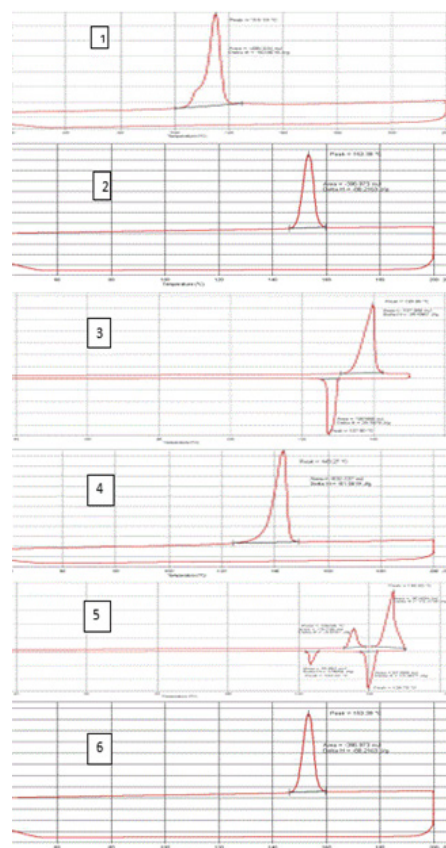


Figure 3: DSC for sebacic acid-KTZ (1), KTZ (2), sebacic acid (3), suberic acid-KTZ (4), suberic acid (5), KTZ (6)

The efforts to co-crystallize ketoconazole with diacids have resulted in an enhancement of the solubility of the active pharmaceutical ingredient (API). This can be beneficial for formulating drug preparations with improved bioavailability. The dosage mentioned for adults, 200 mg daily, is a common dose recommended for ketoconazole. It's important to note that dosages may vary depending on the specific condition being treated and individual patient factors, so it's always best to follow the guidance of a healthcare professional. For infants, the dosage range provided, between 3.3-6.6 mg, suggests that lower doses are required due to their smaller size and differences in metabolism compared to adults.

Considering the positive results obtained, the next logical step would be to utilize oral drug preparations to complete the study. Oral administration is one of the most common and convenient routes for drug delivery, and it allows for systemic absorption through the gastrointestinal tract.

By using appropriate drug preparations for oral administration, you can further investigate the solubility enhancement and evaluate the pharmacokinetics, efficacy, and safety profile of the co-crystallized ketoconazole with diacids. This will provide valuable insights into the potential clinical applications of the formulation and its suitability for oral administration in patients.

#### 4. CONCLUSIONS

In conclusion, this study successfully improved the solubility of KTZ through the crystal engineering technique. Co-crystals of KTZ were formed with co-formers, namely suberic acid and sebacic acid, using the solvent crystallization method. The formation of co-crystals was confirmed through FTIR, DSC, melting point, and water solubility tests. The co-crystals exhibited enhanced solubility, making them suitable for various dosage forms. The solvent crystallization method proved to be a feasible technique that can be applied to other poorly water-soluble molecules

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#### Author's contribution

Data collection, analysis and writing was performed by the author. Corresponding author was supervising and manage the whole work step-by-step with the author.

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