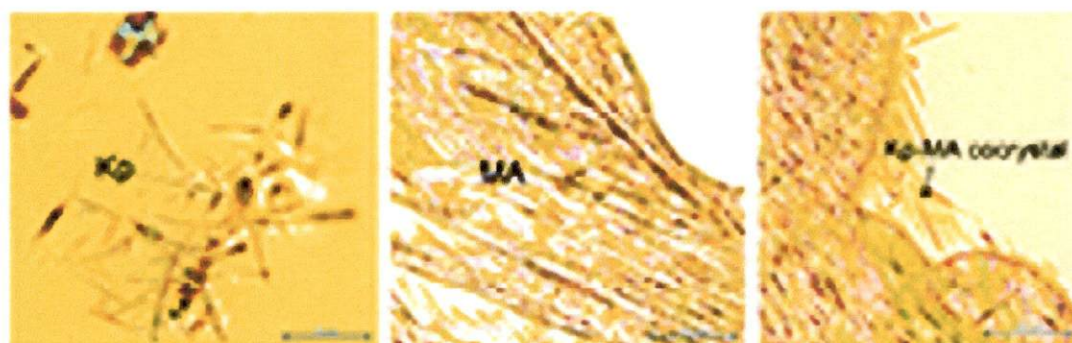


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## Formation of Ketoprofen-Malonic Acid Cocrystal by Solvent Evaporation Method

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### ABSTRACT

The purpose of this work was to explore the formation of ketoprofen-malonic acid cocrystal by solvent evaporation method. Early detection of cocrystal formation was conducted by hot stage microscopy and solid-liquid phase diagram. Cocrystal were prepared by solvent evaporation method by using isopropyl alcohol as solvent. Characterization of cocrystal was done by Powder X-Ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscopy (SEM). The results of hot stage microscopic and solid-liquid phase diagram indicated formation of ketoprofen-malonic acid cocrystal. PXRD and DSC measurements showed stoichiometric ratio of cocrystal ketoprofen-malonic acid (2:1). The ketoprofen-malonic acid cocrystal had melting point at 86.2 °C and unique peaks of PXRD pattern at 2θ of 6.1°, 17.8°, 23.2° and 28.6°. FTIR spectra indicated the formation of cocrystal due to interaction of C=O ketone group of ketoprofen with MA molecule. SEM images show that ketoprofen-malonic acid cocrystal have multi-shaped particles with rough surfaces.

**Keywords:** ketoprofen; cocrystal; hot stage microscopy; solid-liquid phase diagram

### ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui pembentukan kokristal ketoprofen-asam malonat dengan metode penguapan pelarut. Deteksi awal pada pembentukan kokristal dilakukan dengan hot stage microscopy dan diagram fase padat-cair. Kokristal dipreparasi dengan metode penguapan pelarut menggunakan isopropil alkohol sebagai pelarut. Karakterisasi kokristal dilakukan dengan Powder X-Ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy dan Scanning Electron Microscopy (SEM). Hasil hot stage microscopic dan diagram fase padat-cair mengindikasikan adanya pembentukan kokristal ketoprofen-asam malonat. Pemeriksaan PXRD dan DSC menunjukkan perbandingan stoikiometris kokristal ketoprofen-asam malonat (2:1). Kokristal ketoprofen-asam malonat mempunyai titik lebur 86,2 °C dan puncak khas PXRD 2θ pada 6,1°, 17,8°, 23,2° dan 28,6°. Spektra FTIR menunjukkan pembentukan kokristal melalui interaksi gugus C=O dari ketoprofen. Kokristal ketoprofen-asam malonat mempunyai bentuk partikel multi-shaped dengan permukaan kasar.

**Kata Kunci:** ketoprofen; kokristal; hot stage microscopy; diagram fase padat-cair

### INTRODUCTION

Ketoprofen (Kp) is known for its analgesic, anti-inflammation and antipyretic effect. It exhibits low aqueous solubility and dissolution properties [1-2]. These properties cause Kp to have slow absorption and low bioavailability [3]. Kp is a weakly acidic drug (pKa=4.45) and there are no potential counter-ions for salt formation [2].

Cocrystal is a relatively new solid form of active pharmaceutical ingredient that offers an alternative platform in improving physicochemical properties of

active pharmaceutical ingredients [4-5]. Cocrystal is defined as a stoichiometric multi-component system connected by non-covalent interactions where all the components neutral and solid under ambient conditions [6]. Cocrystal can be constructed through interaction hydrogen bonding, pi-stacking, and van der Waals forces [5]. A pharmaceutical cocrystal is composed of an API and an appropriate coformer as carboxylic acids and amides [7]. Co-crystallization of active pharmaceutical ingredient is an opportunity for enhancement of important physicochemical properties of

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an active pharmaceutical ingredient without changing its molecular structure [8].

The most common methods for formation of cocrystal are solution crystallization and mechanical grinding [7,9]. Co-crystallization by solvent evaporation of stoichiometric solutions is a general technique of solution crystallization to prepare cocrystal [7]. In this method, cocrystal components in an appropriate stoichiometric amount are dissolved in a solvent and the solvent allowed to evaporate from solution [6].

In the present study, we explored co-crystallization of Kp by solvent evaporation method. This study aimed to confirm whether Kp was able to form cocrystal with malonic acid (MA) as cofomer. Early detection of cocrystal formation was conducted by hot stage microscopy and solid-liquid phase diagram. Kp-MA cocrystal was prepared by solvent evaporation method by using isopropyl alcohol as solvent. Characterization of cocrystal was done by Powder X-Ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscopy (SEM).

## EXPERIMENTAL SECTION

### Materials

Ketoprofen (Kp) was kindly donated by PT Dexa Medica (Palembang, Indonesia). Malonic acid (MA) and isopropyl alcohol (IPA) were purchased from Merck (Darmstadt, Germany).

### Instrumentation

The instruments used were analytical balance (Precisa ES 225SM-DR), polarized microscope (Olympus BX41), heating stage (Scilogex MS7-H550-Pro), camera (Olympus DP21), differential scanning calorimeter (Rigaku Thermo Plus EVO II), X-ray diffractometer (PANalytical X'Pert-Pro), FT-IR spectrometer (ALPHA Bruker), Scanning Electron Microscope (Hitachi Tabletop Microscope TM 3000), and ion sputter coater (Hitachi E-1045).

### Procedure

#### Hot stage microscopy

HSM method was carried out by polarization microscope equipped heating stage and camera. Powder of MA about 5 mg was placed on object glass and covered with slide. The object glass was then heated, so all powder of MA melted and allowed to recrystallize again. Certain amount of Kp powder was placed at boundary of cover glass and object glass heated again until all powder of Kp melt. The melting of

Kp allowed contact with surface of MA crystals. The contact area was observed with a polarizing microscope to check formation of Kp-MA cocrystals [10].

#### Solid-liquid phase diagram

Certain amounts of powder components were weighted according to desired mole fraction and mixed in a mortar using a spatula. The mixtures were weighed out about 2.5 mg in aluminum pans and analyzed with DSC over the range of 30-200 °C at a heating rate of 10 °C/min. Endothermic peak of DSC curves was plotted against mole fraction of mixtures to obtain solid-liquid phase diagram of Kp-MA [11].

#### Co-crystallization by solvent evaporation method

Kp and MA at different mole ratios (1:1, 1:2 and 2:1) were dissolved in isopropyl alcohol with stirring. The resulting solution allowed to slowly evaporate at an ambient temperature. The resulted cocrystal was crushed and characterized by PXRD, DSC, FTIR, and SEM.

#### Powder X-ray diffraction

Powder X-ray diffraction patterns of samples were collected at room temperature using an X'Pert PRO diffractometer system with Cu K $\alpha$  radiation (1.54060 Å). The voltage and current were set at 40 kV and 30 mA, respectively. Sample was placed in an aluminium sample holder and flatted. The data were collected by a continuous scan over an angle range from 5-50° in 2 $\theta$  at a step size of 0.017° and scanning speed of 10°/min.

#### Differential Scanning Calorimetry

DSC analysis of samples was performed with a DSC which was calibrated for temperature and heat flow accuracy using indium. The samples of 2-3 mg were accurately weighed in hermetic aluminium pans and scanned over the range of 30-250 °C at a heating rate of 10 °C/min.

#### Fourier Transform Infrared Spectroscopy

IR spectra of cocrystals were obtained by an FT-IR spectrometer (ALPHA Bruker). Measurements were recorded over a range 4000-400 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup>.

#### Scanning Electron Microscopy

The morphology and shape of samples were characterized using Scanning Electron Microscope (Hitachi Tabletop Microscope TM 3000) at 15 kV accelerating voltage. All samples were coated with a thin layer of platinum using an ion sputter coater (Hitachi E-1045) before SEM analyses.

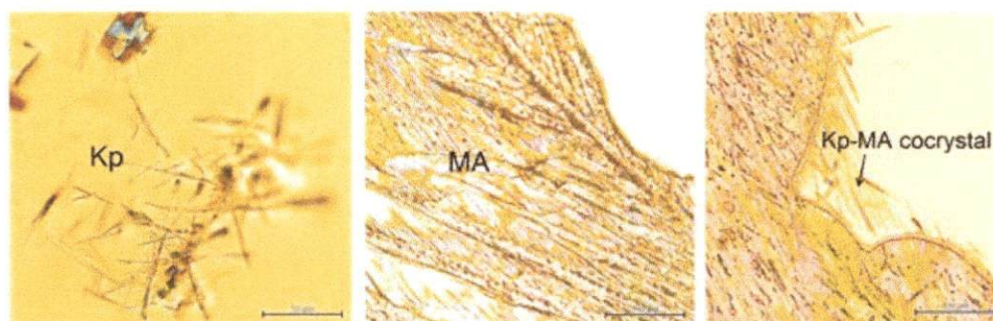


Fig 1. Hot stage microscopy of Kp, MA and Kp-MA cocrystal

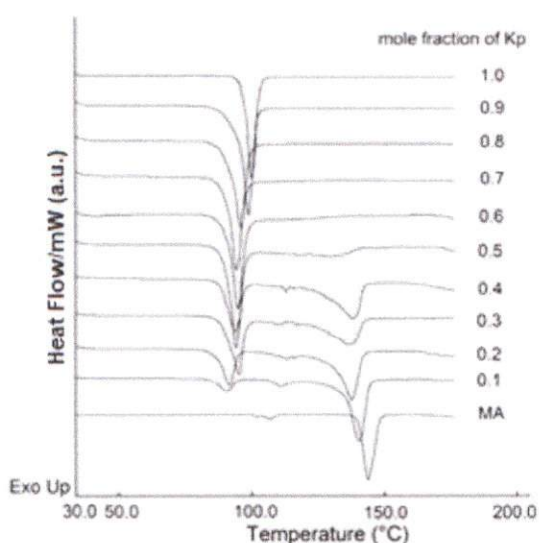


Fig 2. DSC curves of Kp, MA and binary mixtures of Kp-MA at different mole fraction

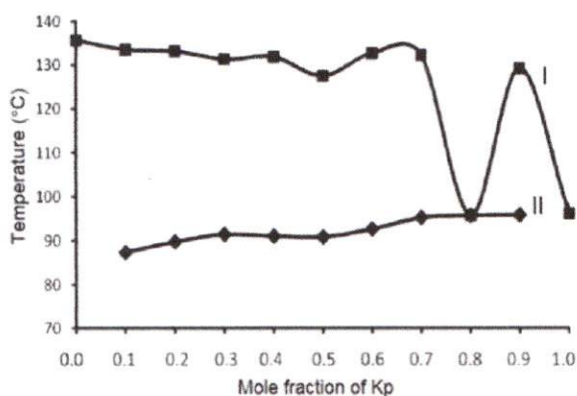


Fig 3. Solid-liquid phase diagram of Kp-MA (I) liquid curve (II) solid curve

## RESULT AND DISCUSSION

### Hot Stage Microscopy

Hot stage microscopy is one of thermal method used to detect the formation of cocrystal [10,12-14]. It

allows distinguishing the melting of a chemical compound from the melting of eutectic mixture. Indication of probability of cocrystal formation obtained new crystal from the melt with different morphology compared crystal of original compounds [14].

The hot stage microscopy of Kp-MA can be seen in Fig. 1. The samples melted partially at each melting point and then allowed to recrystallize. Recrystallization of Kp-MA at contact area shows formation of new crystal that exhibit different forms with individual crystal. The new crystal was estimated as cocrystal of Kp-MA which had a needle shape and larger size than Kp.

### Solid-Liquid Phase Diagram

DSC is used for determining thermal phenomenon of sample over the change of temperature [15]. Solid-liquid phase diagram of binary mixtures can be constructed from DSC measurements of individual component and its mixtures. The DSC curves of Kp, MA and binary mixtures of Kp-MA at different mole fraction are shown in Fig. 2. DSC curve of Kp shows one sharp endothermic peak at 96.1 °C, corresponding to melting point of Kp [16]. The DSC curve of MA has two endothermic peaks at 104.2 and 135.6 that indicate transformation and melting point of MA [17]. The curves of binary mixtures of Kp-MA have peaks related to thermal phenomenon of individual component and its mixtures. The first peak of binary mixtures which appeared at lower temperature was attributed to melting of eutectic mixture and second one, at higher temperature, correspond to melting point of major component [18].

The solid-liquid phase diagram of Kp-MA constructed from the DSC curves is shown in Fig. 3. The binary mixtures of Kp-MA at different mole fraction form a binary eutectic mixture. The eutectic point of binary mixtures at mole fractions of Kp 0.3, 0.4, 0.5 and 0.6 is seen lower than eutectic point of binary mixtures at mole fractions of Kp 0.7, 0.8 and 0.9. These indicate that Kp at mole fractions of 0.3, 0.4, 0.5 and 0.6 form

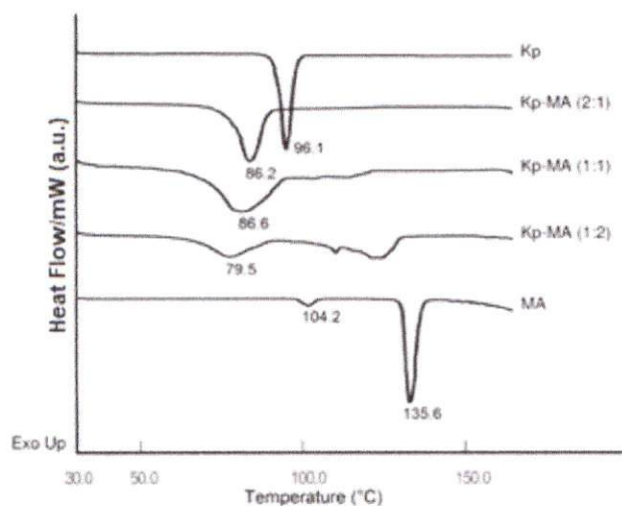


Fig 4. DSC curves of Kp, MA and Kp-MA cocrystal at different stoichiometric ratio

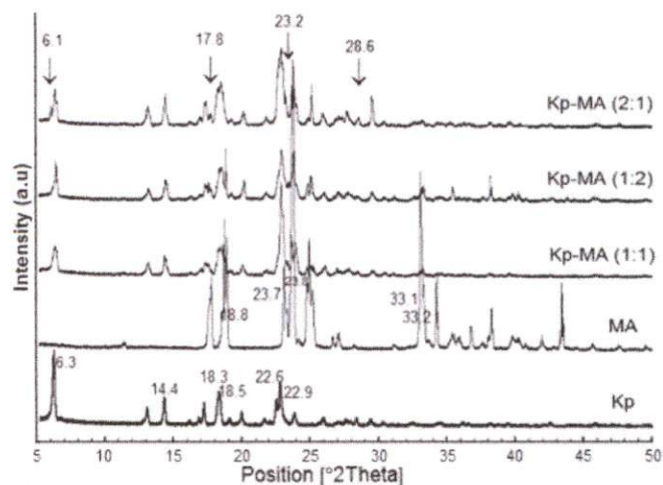


Fig 5. PXRD pattern of Kp, MA and Kp-MA cocrystal at different stoichiometric ratio

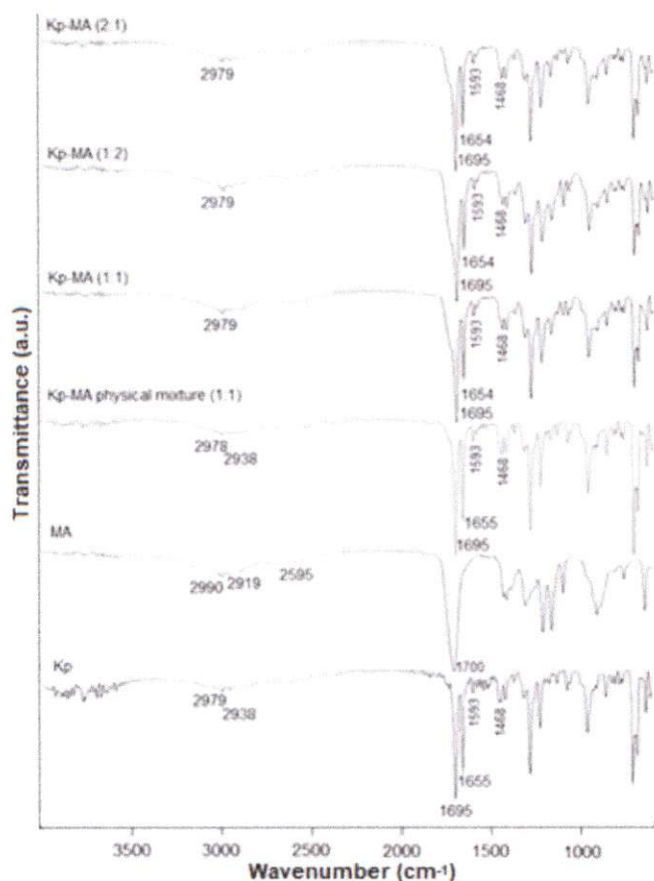


Fig 6. FTIR spectra of Kp, MA, Kp-MA physical mixture and Kp-MA cocrystal

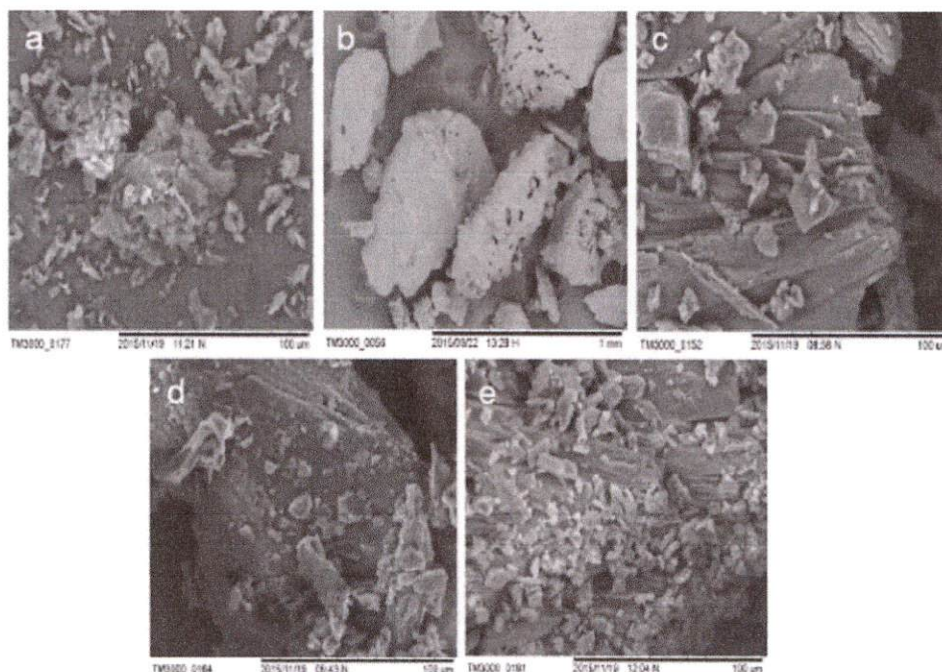
cocrystal with MA, so eutectic point at the mixtures was eutectic point of MA and Kp-MA cocrystal.

### Differential Scanning Calorimetry

DSC measurements were taken to investigate thermal properties of Kp-MA cocrystal. The thermal properties are important to study physicochemical properties of drugs. Formation of cocrystal was evidenced by the appearance of a single endothermic peak attributed to melting of cocrystal phase [19]. The DSC curves in Fig. 4 shows that Kp and MA form cocrystal indicated by an endothermic peak at different temperature with melting point of Kp and MA. Kp-MA cocrystal at mole ratios (1:1), (1:2), and (2:1) each have an endothermic peak at 86.6, 79.5 and 86.2 °C, respectively. It means that the cocrystal have lower melting point than melting point of individual components. DSC curves of Kp-MA cocrystal at molar ratios (1:1) and (1:2) each with two endothermic peaks at 106.7, 118.7 °C and 107.3, 119.4 °C, respectively, indicated transformation temperature and melting point excess of MA. It can be concluded that Kp-MA cocrystal have stoichiometric ratios (2:1).

### Powder X-ray Diffraction

PXRD pattern of a crystalline sample has been in use for fingerprint characterization of crystalline materials. Every crystalline material has unique characteristics in PXRD pattern [20]. The PXRD patterns of Kp, MA and Kp-MA cocrystal at different stoichiometric ratios are shown in Fig. 5. PXRD patterns of Kp exhibited characteristic peaks at  $2\theta$  values of 6.3°, 14.4°, 18.3°, 18.5°, 22.6°, and 22.9°, while MA has characteristic peaks at  $2\theta$  values of 18.8°, 23.7°, 23.8°, 33.1° and 33.2°. Kp-MA cocrystals have unique crystalline peaks of PXRD pattern at  $2\theta$  values of 6.1°, 17.8°, 23.2° and 28.6°. It is



**Fig 7.** SEM images of (a) Kp 1000x (b) MA 100x (c) Kp-MA (1:1) 1000x (d) Kp-MA (1:2) 1000x and (e) Kp-MA (2:1) 1000x

different from the pattern of KTP and MA or physical mixture of them. It indicates formation of new crystalline phase of Kp-MA cocrystal.

#### Fourier Transform Infrared Spectroscopy

FTIR spectroscopy is an important spectroscopic technique in determining the structure conformation of cocrystal. It can distinguish cocrystal from salts when a carboxylic acid is taking part in hydrogen bond formation [21]. FTIR spectra of Kp, MA, physical mixture of Kp-MA and Kp-MA cocrystal are shown in Fig. 6. FTIR spectra of Kp have 2 characteristic absorption peaks of carbonyl peak at 1695 and 1655  $\text{cm}^{-1}$ . It was ascribed to C=O stretching of carboxylic acid and C=O stretching of ketone, respectively [22]. Kp also exhibited O-H stretching at 3400-2400  $\text{cm}^{-1}$  and C=C stretching of aromatic ring at 1593 and 1468  $\text{cm}^{-1}$ . FTIR spectra of MA show O-H stretching at 3400-2400  $\text{cm}^{-1}$  and C=O stretching of carboxylic acid at 1700  $\text{cm}^{-1}$ . The shifts of peaks were not observed in physical mixture of Kp-MA. Meanwhile, the spectra of Kp-MA cocrystal decreased in C=O stretching frequency from 1655  $\text{cm}^{-1}$  to 1654  $\text{cm}^{-1}$ . This shifting indicated C=O ketone group of Kp participate in formation of Kp-MA cocrystal [23].

#### Scanning Electron Microscopy

Morphology and size *have a great influence on* physical properties of cocrystal [24]. SEM images of Kp,

MA and Kp-MA cocrystal are presented in Fig. 7. The SEM analysis revealed that morphology of Kp-MA cocrystal are different from that of individual components. Kp shows plate-shaped particles with a size of approximately 30  $\mu\text{m}$ , while MA has round-shaped particles with a size of approximately 500  $\mu\text{m}$ . Kp-MA cocrystal were multi-shaped particles with rough surfaces.

#### CONCLUSION

Formation of Kp-MA cocrystal by solvent evaporation method had been demonstrated. HSM and solid-liquid phase diagram indicated formation of Kp-MA cocrystal. DSC and PXRD measurements showed Kp and MA form cocrystal with (2:1) stoichiometric ratios. The formation of cocrystal was mainly induced by interaction of C=O ketone group of Kp. Kp-MA cocrystal have multi-shaped particles with rough surfaces.

#### ACKNOWLEDGEMENT

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