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Quercetin-maleic acid co-crystal engineering using solvent evaporation to ıncrease quercetin solubility

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ABSTRACT: This study aims to produce quercetin-maleic acid cocrystals with an increase in solubility properties. The preparation of quercetin-maleic acid cocrystal (CQAM) was carried out by solvent evaporation method using ethanol as a solvent. The quercetin-maleic acid cocrystal preparation was formed with two different stoichiometric ratios, namely quercetin-maleic acid (1:1 and 1:3). Solubility tests on quercetin-maleic acid cocrystals were carried out in 50 mL citrate buffer pH 5 at 37±0.5°C. The characterization tests used were DSC, PXRD, FTIR, and SEM, while for the evaluation, solubility tests were carried out. The results of DSC, PXRD, FTIR and SEM showed the formation of a new solid with the type of crystalline solid, so it was concluded as a cocrystalline solid. The results of the solubility test showed that the solubility value of quercetin-maleic acid cocrystal (1:1 and 1:3) was greater than that of pure quercetin. Solubility result of pure quercetin, CQAM 1:1 and CQAM 1:3 were 0.545 ± 0.0351 mg/L; 2.237 ± 0.0258 mg/L; and 6.352 ± 0.0258 mg/L, respectively. Quercetin–maleic acid cocrystals (1:1) and (1:3) experienced an increase in solubility of 4.11 and 11.65 times, respectively, from pure quercetin.

KEYWORDS: Cocrystal; Quercetin; Maleic Acid; Solvent Evaporation; Solubility.

1. INTRODUCTION

Quercetin (3,3',4',5,7-pentahydroxy flavone) is one of the most common flavonoid compounds found in food and in almost all plant kingdoms in the form of glycosides, such as berries, onions, cherries, grapes, broccoli, fruit. oranges, green tea, coffee, red wine, capers, and some others [1]. According to the Biopharmaceutical Classification System (BCS), quercetin is classified in BCS class II compounds and included in Generally Regarded as Safe (GRAS) [2]. Quercetin has an very poor solubility in water [3] of 0.01 mg/mL at 25°C [4] which results in low quercetin bioavailability. This poor water solubility characteristic of quercetin significantly reduces its absorption in the gastrointestinal tract (GIT), resulting in decreased bioavailability and failure to achieve the desired therapeutic effect with oral administration [4].

Pharmaceutical cocrystals are solids that contain two or more different molecules in certain stoichiometric ratios to form new crystals with better solubility in water than pure compounds [5-7] involving non-covalent bonds such as hydrogen bonds, Van der Waals forces, and π-π* interactions [8]. The formation of a cocrystal system has the potential to improve physicochemical properties such as solubility, dissolution rate, bioavailability and physicochemical stability of an active drug substance without affecting its pharmacological activity [9,10]. The advantage of this technique is that it can be applied to all types of drugs, whether alkaline, acidic, ionized or non- ionized, and can change the physical properties of the material due to changes in the internal crystal structure without being accompanied by changes in the pharmacological activity of the drug [1]. Several studies to improve the physicochemical properties of quercetin have been carried out, such as increasing solubility through the formation of inclusion complexes with β-Cyclodextrin [11], increasing absorption and bioavailability through making nanophytosome preparations [12], making quercetin in the form of nanocrystals [9,13], making quercetin–PVP K-25 solid dispersion [3], increasing the dissolution rate of quercetin by nanofabrication, complexation, and solid dispersion [8], increasing the dissolution rate in vitro from quercetin-acid cocrystals malonate [4].

The aim of this research is to form quercetin–maleic acid cocrystals at ratios of 1:1 and 1:3 by solvent evaporation method which is expected to improve physicochemical properties such as solubility properties and dissolution rate. The next stage, the cocrystals formed, were characterized using DSC, PXRD, FTIR and SEM as well as solubility and dissolution tests [11].

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2. RESULTS AND DISCUSSION

2.1 Determination of Coformers

The computational results obtained are visualization of predictions of hydrogen bonds that occur between quercetin and each coformer and minimize energy values: succinic acid (-28.0946 kcal/mol), maleic acid (-31.6180 kcal/mol), malonic acid (-22.6012 kcal/mol), citric acid (-31.2273 kcal/mol), and adipic acid (-12.6277 kcal/mol). The results can be seen in Figure 1.

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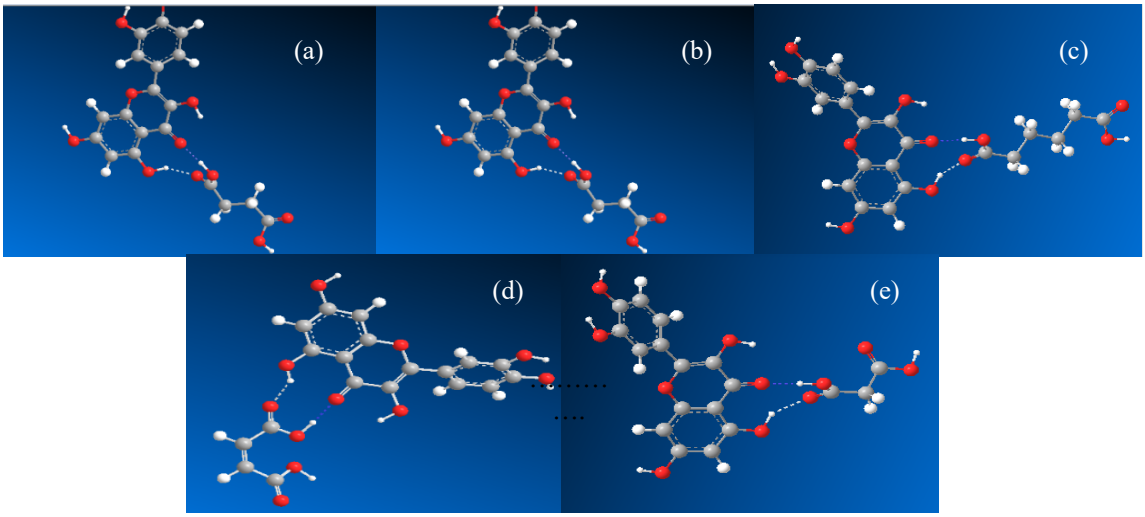
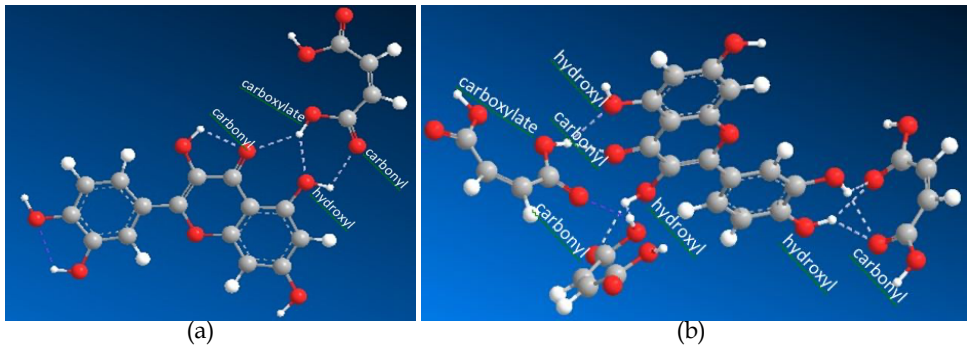


Figure 1. Prediction of hydrogen bonds formation between quercetin and the co- formers: citric acid, (b) succinic acid, (c) adipic acid, (d) maleic acid, (e) malonic acid

The computational results between quercetin and maleic acid (1:1) can be seen in Figure 2a showing the occurrence of hydrogen bonds between the carbonyl group on ring C of quercetin and the carboxyl group of maleic acid. Hydrogen bonding also occurs in the hydroxyl group of the A ring of quercetin with the carboxyl and carbonyl groups of maleic acid. Figure 2b at a ratio of 1:3 shows the presence of hydrogen bonds between the hydroxyl group on ring A of quercetin and the carboxylic group of maleic acid; hydrogen bonding between the hydroxyl group on ring C of quercetin and the carbonyl group of maleic acid and hydrogen bonding between the hydroxyl group on ring B of quercetin and the carbonyl group of maleic acid. Based on the minimized energy obtained from several coformers, the lowest energy minimized result was obtained by maleic acid coformers, which was -31.618 kcal/mol (Figure 1). The minimized energy value states the amount of energy required for a compound to undergo interactions, especially hydrogen bonds. The lowest energy minimize indicates the most stable hydrogen bonding [13]. Therefore, in this study, the maleic acid coformer was selected in the preparation of quercetin cocrystals.



(a) (b)

Figure 2. Computational results of hydrogen bonds between molecules of quercetin and maleic acid with ratios of (a) 1:1 and (b) 1:3

2.2. Quercetin-Maleic Acid Cocrystal Characterization.

2.2.1. Differential scanning calorimetry (DSC).

The DSC thermogram in Figure 3. shows the presence of different endothermic peaks between quercetin, maleic acid and quercetin-male.ic acid cocrystal (CQAM) at two stoichiometric ratios. In the thermogram of quercetin-maleic acid cocrystal with a ratio of 1:1, a new thermogram peak was formed, namely at a melting temperature of 304.45°C, while the melting temperature of quercetin (322.17°C) and maleic acid (141.82°C) was not visible on the thermogram of quercetin-acid maleic cocrystal. Quercetin-maleic acid cocrystal with a ratio of 1:3 indicated the formation of a new thermogram peak at a melting temperature of 301.93°C. Quercetin-maleic acid cocrystal has a melting point that is between the melting points of its constituent materials.

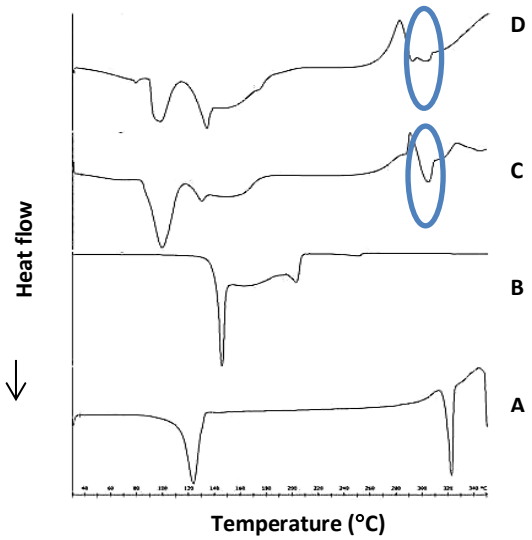


Figure 3. DSC thermogram profile (A) quercetin (B) maleic acid (C) CQAM 1:1 (D) CQAM 1:3

In cocrystals of quercetin-maleic acid r.atio 1:1 and 1:3 also seen the endothermic peak in the 100-120°C region which indicates a dehydration process in the cocrystals. This is because the quercetin used in this study is quercetin in the form of dihydrate. In the 1:3 ratio quercetin-maleic acid cocrystals, endothermic peaks are not too sharp in the 100-120°C region, this may be related to the molar amount of maleic acid which is more bound to quercetin, causing it to be detected but not as sharp as the stoichiometric ratio cocrystal peaks 1:1 on thermal analysis.

2.2.2. Scanning Electron Microscopy

The quercetin-maleic acid cocrystal in Figure 4 shows a different crystal habit with the two constituent components. The 1:1 ratio cocrystal has an uneven acicular shape, while the 1:3 ratio cocrystal has a needle crystal shape with a smoother surface such as small fibers than the 1:1 ratio. Scanning Electrone Microscopy results shows that cocrystals of quercetin-maleic acid ratio 1:1 and 1:3 have different crystal shape and habit than pure quercetin and maleic acid. Cocrystal formation can cause change in crystal habit, because there is a new crystallins forms in cocrystal, as an effect of hydrogen bond between quercetin and maleic acid [14].

2.2.3. Powder X-Ray Diffractometer

Characterization using Powder X-Ray Diffraction (PXRD) was carried out to detect the crystal form of a mixture that was displayed in a diff.raction pattern. The diffraction peak formed is a reflection of the diffraction pattern of the crystal lattice structure formed [11]. The quercetin-maleic acid cocrystal sample (1:1) produced a new crystal form that differed from the two constituent materials, with a specific 2θ diffraction peak at an angle: 13.6194^{o}; 15.8396^{o}; 17.7519^{o}; and 26.9030^{o} (Figure 5). New crystallins also appeared in 1:3 ratio quercetin-maleic acid cocrystal samples with different diffraction peaks of the two starting materials: 13.4608^{o}; 15.7649^{o}; 17.3694^{o}; and 26.9403^{o}.

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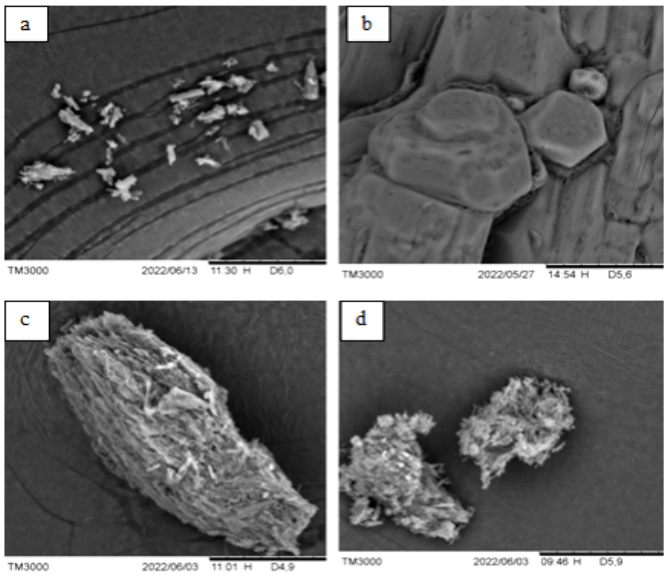

Figure 4. SEM at 1500x magnification (a) quercetin (b) maleic acid (c) CQAM 1:1 (d) CQAM 1:3

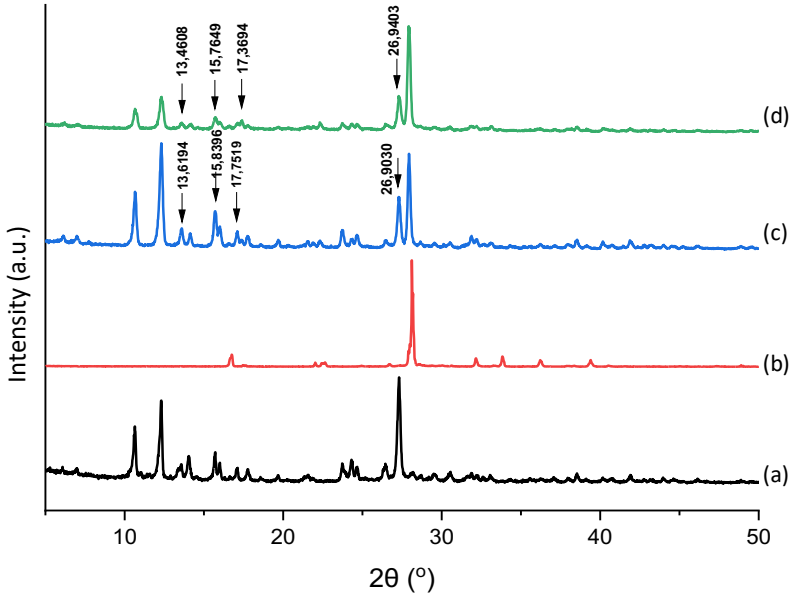

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Figure 5. Diffractogram of PXRD (a) quercetin (b) maleic acid (c) CQAM 1:1 (d) CQAM 1:3

2.2.4. Fourier Transform Infrared Spectroscopy

Analysis of sample characterization with FTIR is characterized by the identification of functional groups and molecular interactions in the sample. The infrared spectra of quercetin, maleic acid, quercetin-maleic acid cocrystal (1:1) and quercetin-maleic acid cocrystal (1:3) can be seen in Figure 6. The wave number of the OH group in quercetin shifted from 3412 cm^{-1} to 3395.27 cm^{-1} in the cocrystal infrared spectra of 1:1 ratio and 3400.91 cm^{-1} in the cocrystal spectra of 1:3 ratio. The shift in the wave number of the C=O group in quercetin was also seen from 1665 cm^{-1} to 1705.85 cm^{-1} in the 1:1 ratio quercetin maleic acid cocrystal and 1705.86 cm^{-1} in the 1:3 ratio quercetin-maleic acid cocrystal. This indicates a shift in the wave numbers of the O-H and C=O groups between pure quercetin and quercetin-maleic acid cocrystals. The occurrence of intervention in the wave numbers of the OH and C=O groups indicates the interaction between quercetin and maleic acid through hydrogen bonds, thus forming supramolecular heterosynthone cocrystal quercetin-maleic acid [13].

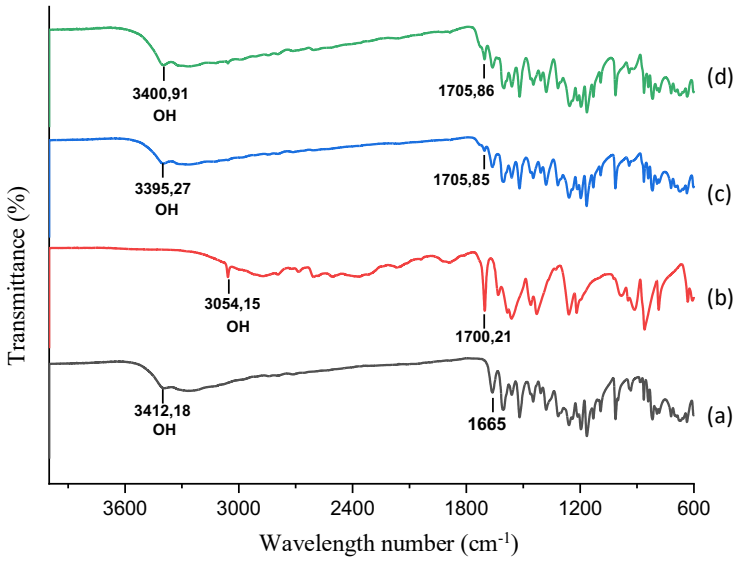

Figure 6. Infrared spectra (a) quercetin (b) maleic acid (c) CQAM 1:1 (d) CQAM 1:3

2.3. Solubility Test Results

The test was carried out on citrate buffer pH 5 with a saturation time of 180 minutes[16]. The test was carried out with three replications on pure quercetin samples and quercetin-maleic acid cocrystals in both ratios. The results of the solubility test showed that the quercetin-maleic acid cocrystals in both ratios had higher solubility compared to the solubility of pure quercetin. This can be seen in table 1.

The increase in solubility of quercetin-maleic acid cocrystals is caused by each component of the active ingredient quercetin with maleic acid coformers formed noncovalent interactions, namely hydrogen bonds in the OH group of quercetin with the OH group of maleic acid, this has been proven by synthon in computational analysis methods. The difference in stoichiometric ratio affects the solubility of cocrystal formation. In this study, cocrystal quercetin-maleic acid ratio 1:3 showed an increase in solubility (6.352 mg/L ± 0.0258) which was greater than the 1:1 ratio (2.237 mg/L ± 0.0258).

Table 1. Solubility of quercetin, quercetin- maleic acid cocrystal at two stoichiometric ratios in citric buffer solvent pH 5 (37±0.5ºC, n=3)

Solubility (mg/L)			
Replication	**Quercetin**	**CQAM (1:1)**	**CQAM (1:3)**
1	0.5565	2.2091	6.3575
2	0.5734	2.2428	6.3744
3	0.5059	2.2597	6.3238
Average ± SD	0.545 ± 0.0351	2.237 ± 0.0258	6.352 ± 0.0258

Based on the results of the characterization and solubility test of quercetin and quercetin-maleic acid cocrystals, it can be stated that each parameter carried out in the characterization test includes thermal analysis, crystallinity, crystal morphology, and transitional vibration, able to explain the solubility that occurs in quercetin-maleic acid cocrystals with solvent evaporation method in ethanol solvent.

3. CONCLUSION

Based on the research that has been done, it has been proven that quercetin and maleic acid coformers can be formed into quercetin-maleic acid cocrystals by solvent evaporation method. The formation of cocrystals showed that there were differences in physicochemical characteristics that led to an increase in solubility and dissolution rate compared to pure quercetin. In the 1:1 ratio quercetin-maleic acid cocrystal, the solubility increased by 4.11 times, while the 1:3 ratio quercetin-maleic acid cocrystal increased 11.65 times from pure quercetin.

4. MATERIALS AND METHODS

4.1 Reagents and Materials

The materials used in this research were quercetin dihydrate pro analysis (Tokyo Chemical Industry®, Jepang), maleic acid (Merck, Jerman), ethanol pro analysis (Merck, Jerman), and ethanol pro analysis (Emsure®, Merck, Germany).

In this research, the tools used include: UV-VIS Spectrophotometer (Thermo Scientific Genesys 10S), Differential Scanning Calorimeter (Thermo plus EVO DSC 8230), Powder X-Ray Diffractometer (Panalytical Xpert Pro PW3373/00), Fourier Transform Infrared Spectrophotometer (Bruker FTIR Alpha II), Scanning Electron Microscope (Hitachi TM3000), analytical balance (Precisa ES 225 SM-DR), magnetic stirrer (ika c-Mag HS-7), 0.45 µm filter membrane.

4.2 Methods

4.2.1. Prediction of Cocrystal Formation (Computational Approach)

Computational approach with Chemdraw Ultra 12.0 software from several candidate coformers. Some of the coformers that can be used with a computational approach include: maleic acid, citric acid, malonic acid, succinic acid and adipic acid. The computational results are visualization of predictions of hydrogen bonds that occur between quercetin and each coformer and minimize energy. The condition for determining the chosen coformer from the computational results is a low minimized energy value. This shows that the smaller the minimized energy value indicates the best ability of a compound to form hydrogen bonds [12].

4.2.2. Preparation of CQAM

The initial stage of cocrystal preparation is by weighing the active ingredient quercetin and maleic acid coformer according to the stoichiometric ratio. The weighing results are put into a glass beaker separately and then dissolved in pro-analytical ethanol solvent according to the solubility of each ingredient. The active ingredient that has been dissolved is poured into the cup at the same time as the coformer based on a predetermined ratio The cocrystal solution was stirred at 100 rpm on a hotplate using a magnetic stirrer until the solvent evaporat.ed completely and produced the desired cocrystal powder. The cocrystals formed were sieved through a 60 mesh. The resulting cocrystal is stored in a desiccator.

4.2.3. Characterization of CQAM using Differential scanning calorimetry

Characterization using DSC is carried out to measure the amount of heat absorbed during the transition phase and detect changes in the sample transition, both endothermic and exothermic. This characterization was carried out by inserting 5 mg of the sample into an aluminum pan, then thermal analysis by means of a DSC at a temperature range of 30-350°C with a heating speed of 10°C per minute [16].

4.2.4. Characterization od CQAM using Scanning Electron Microscopy

Sample characterization using SEM (Hitachi TM3000) in the form of morphological, microscopic and size data from the tested samples. A sample of 2 mg is placed on a stub specimen that has been glued. The sample was coated for 20 seconds using platinum with an ion sputter (Hitachi E-1045), then inserted into the SEM holder base. The SEM. device is set to a voltage of 15kV and a checking current of 12 mA [12].

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4.2.5. *Characterization of CQAM using Powder X-Ray Diffractometer*

This tool is equipped with a CuKα1 radiation source (λ = 1.542 A^o) which aims to determine the crystal lattice structure. The characterization steps with PXRD were carried out as follows: the sample powder was inserted into the sample holder cavity of the X-ray diffractometer and leveled with a spatula, the X-ray diffractometer voltage was set at 40 kV, the current was 30 mA and the scanning speed was 2θ = 10^o/min with a range of 2θ at 5-50^o [11].

4.2.6. Characterization of CQAM using Fourier Transform Infrared Spectroscopy

FTIR test was used to identify functional groups and the presence of intermolecular interactions between quercetin molecules and coformers. The sample is weighed as much as 5 mg and put on the FTIR (Alpha Bruker) sample board. The spectrum was collected in the range of 400 to 4000 cm^{-1} [12].

4.2.7. Determination of Quercetin Solubility

Weighing pure quercetin at 20 mg. The sample was put into a solubility test chamber bottle containing 50 ml of pH 5 citrate buffer solvent. Then stirred with the help of a magnetic stirrer at a speed of 100 rpm at a temperature of 37°C ± 0.5°C. Sampling of 5 ml was carried out at 180 minutes. The sample was left for 5 minutes in the chamber, then filtered using whattman paper measuring (0.45µ) with the help of an injection

syringe. The filtering results determined the absorbance at the maximum wavelength of quercetin using a UV-Vis spectrophotometer. Solubility test was carried out on 1:1 and 1:3 cocrystal samples with 3 times replication in citrate buffer pH 5.0 [13].

4.2.8 Statistic analysis

The software used is SPSS 22.0 for windows. Sample testing with Shapiro-Wilk (if the significance> 0.05 then it is said to be a normal distribution). Next, test for homogeneity (if the significance is > 0.05 then it is said that the sample being tested is homogeneous). Testing this data using one- way ANOVA with a 95% confidence level. The difference is considered significant if the value of p <0.05, otherwise if p> 0.05 then the difference is considered not significant [11]. If there is a significant difference, then the next stage is a post hoc test (LSD).

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