Tropical Journal of Pharmaceutical Research April 2019; 18 (4): 697-702
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i4.3

Original Research Article

Co-crystallization of quercetin and isonicotinamide using solvent evaporation method

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Sent for review: 16 October 2018 *Revised accepted: 16 March 2019*

Abstract

Purpose: *To obtain quercetin-isonicotinamide co-crystal (CQINA) with improved physicochemical and in-vitro dissolution characteristics.*
Methods: *Co-crystallization of quercetin (Q) and isonicotinamide (INA) in molar ratio of 1:1 was performed using solvent evaporation method with the addition of 50 mL of ethanol (99.9%, v/v). The resultant solution was thoroughly mixed and stirred at room temperature for 48 h to slowly evaporate the solvent until CQINA was obtained. The co-crystal phase was characterized using differential scanning calorimetry (DSC), powder x-ray diffractometry (PXRD), scanning electron microscopy (SEM), and fourier transform infrared (FTIR) spectroscopy. In-vitro dissolution was performed by USP method II in 900 mL citrate buffer (pH 5.0 ± 0.05), with stirring at 100 rpm and at 37 ± 0.5 °C.*
Results: *Computational approach predicted the formation of hydrogen bonds between Q and co-formers used, and the interaction involved minimum energy. In CQINA thermogram, a new endothermic peak was formed with a melting point of 255.26 °C, while Q (314.85 °C) and INA (156.62 °C). Images from DSC, PXRD, SEM and FTIR showed that the crystal habits and morphologies of the CQINA differed from those of the original components. There was an improvement in the dissolution profile of CQINA, when compared with those of the original components.*
Conclusion: *Q and INA subjected to solvent evaporation result in the formation of a CQINA with different crystal habit, which possess physicochemical characteristics different from those of its constituents. Modification of Q crystals in CQINA increases its in vitro dissolution, making it a potential pharmaceutical agent.*

Keywords: *Quercetin, Cocrystal, Isonicotinamide, Solvent evaporation, Crystalline, Dissolution*

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INTRODUCTION

Quercetin is a flavonoid possesses antioxidant, anti-cancer, anti-viral, anti-obesity, anti-bacterial and anti-inflammatory properties [1-5]. It is classified as a class II compound according to Biopharmaceutics Classification System (BCS) and is included in the Generally Recognized as Safe (GRAS) material [6,7]. Quercetin is highly soluble in ethanol, and dimethyl sulfoxide (DMSO) and sparingly soluble in water [8,9]. This poor aqueous solubility characteristic significantly decreases its absorption in the gastrointestinal tract (GIT), thus lowering its bioavailability and

resulting in failure to attain the desired therapeutic effect on oral use [10].

Co-crystallization is a strategy that can be used to increase the solubility of a compound. Pharmaceutical co-crystals contain two or more distinct molecules assembled to create a new crystal with good water solubility, relative to those of the pure compounds. Co-crystals are solid that are crystalline, single phase materials composed of two or more different molecular or ionic compounds generally in stoichiometric ratios which are neither solvates nor simple salts [11]. Interactions involved in co-crystal formation are hydrogen bonds, Van der Waals forces, and π-π* interactions [12].

The aim of this study was to determine possible interaction between quercetin and several co-formers through a computational approach, and obtain quercetin-isonicotinamide co-crystal (CQINA) with increased physicochemical and *in vitro* dissolution characteristics. The characterization of co-crystal formed was done using PXRD, DSC, SEM, FTIR, and in-vitro dissolution test to determine the changes in quercetin physicochemical characteristics.

EXPERIMENTAL

Materials

Quercetin and INA were obtained from Tokyo Chemical Industry (Japan), while absolute ethanol was purchased from Sigma-Aldrich (France). Differential scanning calorimeter was a product of Mettler (Switzerland) and powder X-ray diffractometer was obtained from Philip X'Pert (Netherlands). Scanning electron microscope was purchased from Hitachi Co., Ltd. (Japan); infrared (IR) spectrophotometer was a product of Jasco (Japan), while UV-Visible spectrophotometer was purchased from Genesys (USA).

Prediction of co-crystal formation

Computational approach

This computational approach was performed using *Marvin Sketch 5.2.5.1* and *ChemBioDraw Ultra 12.0* to visualize hydrogen bond interaction between quercetin and the co-formers. Computational approach was also carried out to determine total energy, which is measure of the ability of quercetin and the co-formers to form hydrogen bonds. The co-formers used included: tartaric acid, maleic acid, mannitol, saccharin, nicotinamide, isonicotinamide (INA), lysin, and arginine.

Crystallization reaction

The formation of new crystals through physical interactions between the two components was carried out using heat contact method [13]. Crystalline behavior of each component and the results of quercetin and INA crystal physical interactions were observed using a polarized light microscope.

Binary phase diagramming

Quercetin-INA binary phase diagram was drawn by observing the endothermic peak formed in quercetin-INA physical mixture. The endothermic peak was obtained from the profile of DSC thermogram.

Preparation of CQINA

The CQINA was prepared using solvent evaporation method [13]. Quercetin and INA (1: 1 molar ratio) were dissolved in 50 mL absolute ethanol (99.9% v/v). The resultant solutions was thoroughly mixed and stirred at room temperature for 48 h slowly evaporate the solvent until CQINA was obtained.

Characterization of CQINA

Thermal analysis

Portions of CQINA was put in an aluminum pan, and subjected to DSC at heating speed of 5 ^{o}C/min within a temperature range of 30–350 ^{o}C. The melting point of the sample was determined from the profile of the thermogram.

Crystallinity test

Analysis of CQINA crystalline phase was carried out using powder X-ray diffraction with the following conditions: Cu monochromator, 40 kV voltage, current of 30 mA, and 0.2 inch slit width. Data was collected in scanning mode of 0.2^{o} - 0.5^{o} per min in 2θ range of 5^{o} - 40^{o} at room temperature.

Crystal morphology

Characterization of crystal morphology was performed using SEM. Powdered samples of CQINA were put in sample holder and coated with gold to a thickness of 10 nm. They were then observed at various magnifications at a regulated working voltage of 20 kV and a current of 12 mA. Comparison of CQINA crystal morphology was made with those of quercetin and INA crystals.

Vibrational transition

Vibrational transitions were observed and analyzed using IR spectrophotometer. The samples were mixed with KBr crystal (1:100), homogenize and then subjected to pressing at 20 psi. The spectra were measured using Jasco-4200 FTIR spectrometer and observed in the absorption band or wavelength of 4000 - 450 cm^{-1}. The resultant CQINA FTIR spectra were compared with those of quercetin and INA.

Dissolution test

This was performed on quercetin, physical mixture of quercetin-INA, and CQINA obtained from solvent evaporation. Each weighed sample was equivalent to 20 mg quercetin. *In vitro* dissolution was performed using USP method II in 900 mL citrate buffer (pH 5.0 ± 0.05) stirred at 100 rpm and temperature of 37 °C. Aliquots of the samples (5.0 mL) were withdrawn intermittently at intervals of 5, 10, 15, 30, 45, and 60 min, and filtered with a 0.45 µm cellulose filter membrane [14]. Absorbance of each sample was read in a UV-Visible spectrophotometer at maximum wavelength.

Statistical analysis

Data are expressed as mean ± SD, and statistical analysis was performed using SPSS (18.0). One-way analysis of variance (ANOVA) was performed for multiple comparison. Statistical significance showed with p value < 0.05. Winplotr was used for processing and analyzing powder x-ray diffraction data, while Marvin Sketch 5.2.5.1 and ChemBioDraw Ultra 12.0 were used for visualizing and predicting hydrogen bond interactions between quercetin and the co-formers.

RESULTS

Outcome of CA

Computational approach predicted the formation of hydrogen bonds between quercetin and co-formers used, and interaction involved minimum energy: tartaric acid (66.3288 kcal/mol), maleic acid (35.6241 kcal / mol), mannitol (1052.85 kcal/mol), lysine (53.6929 kcal/mol), arginine (85.3495 kcal/mol), saccharin (44.1072 kcal/mol), nicotinamide (41.5672 kcal/mol), and isonicotinamide (20.0104 kcal/mol). These results are shown in Figure 1.

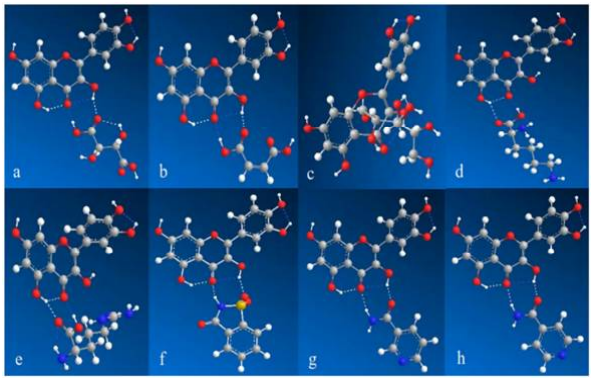

Figure 1: Prediction of hydrogen bonds formation between quercetin and the co-formers: (a) tartaric acid, (b) maleic acid; (c) mannitol; (d) lysine; (e) arginine; (f) saccharin; (g) nicotinamide; (h) isonicotinamide (INA)

Crystal interaction

Physical interactions between quercetin and INA are shown in Figure 2. The shapes of the new crystals formed were indicative of quercetin co-crystals. Polarization microscopy revealed the presence of new crystalline behavior different from those of its original components.

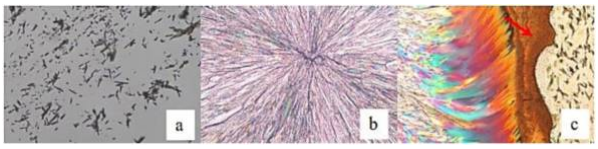

Figure 2: Photomicrographs showing crystal behavior as revealed by polarizing light microscope (x 200). (a) Quercetin; (b) INA; and (c) Quercetin-INA.

Binary phase diagram of quercetin-INA

Binary phase diagram was drawn to confirm the existence of physical interactions between quercetin and INA, forming co-crystal. As shown in Figure 3, there was formation of new endothermic peaks and congruent melting points at 260.62 °C.

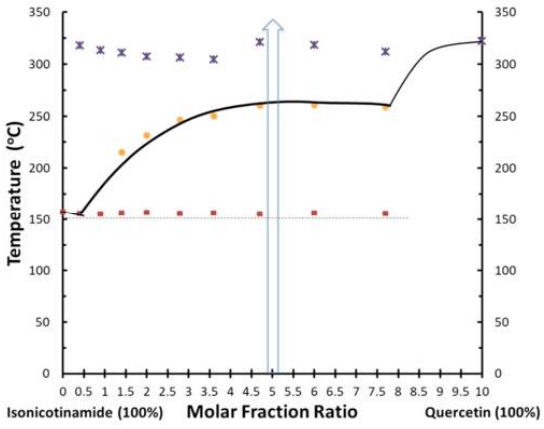

Figure 3: Diagram of quercetin-INA binary system

DSC thermograms

As shown in Figure 4, DSC thermograms revealed differences in endothermic peaks between quercetin, INA, and CQINA. In CQINA thermogram, a new endothermic peak was formed at a melting point of 255.26 °C, while quercetin (314.85 °C) and INA (156.62 °C) endothermic peaks were not observed in CQINA thermogram.

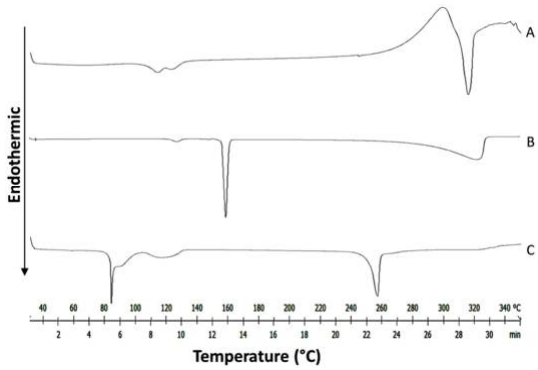

Figure 4: Profiles of DSC thermograms (A) Quercetin; (B) INA; and (C) CQINA

Powder x-ray diffractograms

As shown in Figure 5, there were differences in diffraction peaks between quercetin, INA, and CQINA. New and distinct interference peaks were formed in CQINA at an angle of 2θ: 6.19° (25.38 %); 7.72° (11.29 %); 17.18° (22.39 %); 23.94° (21.87 %); and 24.04° (21.92 %). These differences were indicative of co-crystal formation, since new crystalline phases were produced.

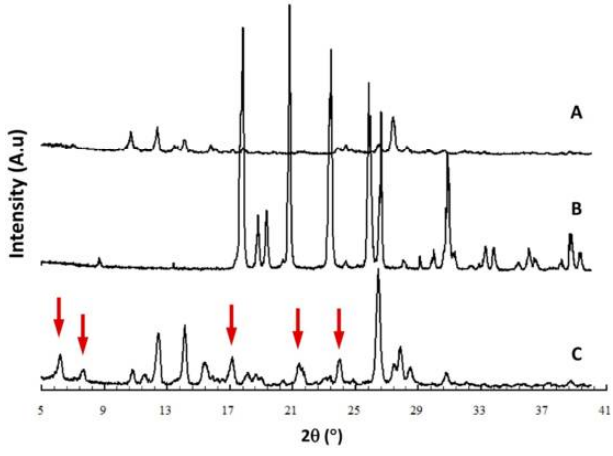

Figure 5: Powder X-ray diffractograms of (A) Quercetin; (B) INA; and (C) CQINA

Crystal morphology

Quercetin exhibited crystalline habit with prism-like shape (size of about 20 – 100 µm), while INA exhibited crystal habit in the form of an angular shape with size range of 100 – 300 µm. The crystal habit of CQINA was different from those of its original components, and was needle-shaped (Figure 6).

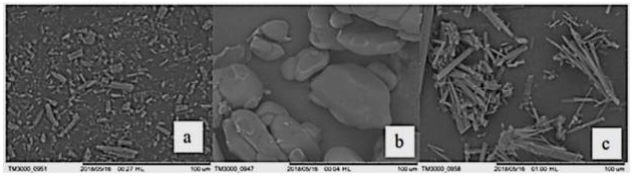

Figure 6: Photomicrographs of samples as revealed in SEM (x 1000). (a) Quercetin; (b) INA; and (c) CQINA

FTIR spectra

The FTIR spectrum of CQINA was different from those of its original components. Quercetin spectrum revealed OH -group at 3413.52 cm^{-1} and C = O group at 1667.62 cm^{-1}. The spectrum of INA revealed NH_2 group at 3371.33 cm^{-1} and 3186.38 cm^{-1}, and C = O groups at 1668.39 cm^{-1}. The spectrum of CQINA also revealed OH – group shift from 3319.51 to 3215.52 cm^{-1} and C = O group stretching at 1688.47 cm^{-1} and 1653.46 cm^{-1}. The NH_2 group in INA was not obvious in CQINA spectrum (Figure 7).

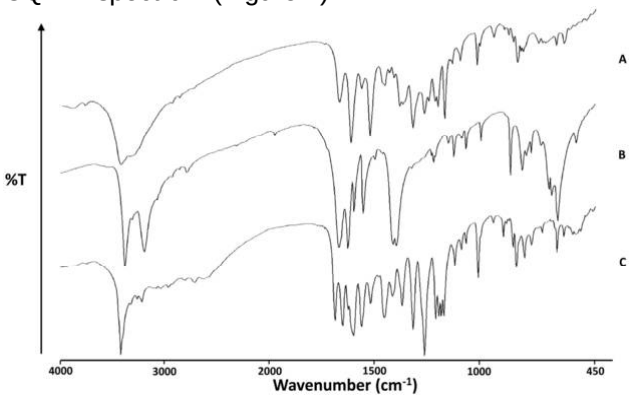

Figure 7: FTIR spectra of samples. (A) Quercetin; (B) INA; and (C) CQINA.

Dissolution profiles

Quercetin, quercetin-INA physical mixture, and CQINA dissolution profiles are shown in Figure 8. The dissolution profile of CQINA revealed an improvement when compared with those of its original components. After 60 min, the percentage dissolution of quercetin and INA were 76.7 ± 1.15 and 75.8 ± 0.19, respectively. The *in-vitro* dissolution of CQINA was 83.3 ± 1.38 % within 60 min.

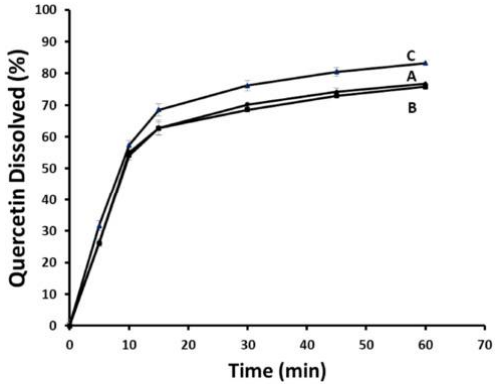

Figure 8: Dissolution profiles of samples. (**A**) Quercetin; (**B**) Quercetin-INA physical mixture; and (C) CQINA

DISCUSSION

In the present study, CA was employed to predict the formation of co-crystal between quercetin and eight potential compounds as co-formers. Computational approach (CA) can predict the most stable interaction form of supramolecular heterosynthone assembly of quercetin and the co-formers. The results showed that INA had the lowest minimized energy, indicative of the most stable form of interaction.

Polarization microscopy revealed the formation of new crystals at the meeting point between quercetin and INA crystals, whose shape and color were different from those of its original components. This result suggests that the new crystal shape may be that of a co-crystal.

Results of DSC revealed several endothermic peaks which formed a congruent system. The formation of a new endothermic peak which was a congruent melting point or co-crystal melting point served as an initial indicator of the formation of a co-crystal. Studies have shown that in some drug combinations, the formation of several endothermic peaks is an early indication of the formation of a co-crystal from the physical mixture [15].

The thermal properties of CQINA as revealed by DSC showed the presence of new endothermic peaks with melting points that were between those of quercetin and INA. This implies the formation of a new solid phase with different thermal properties from those of the original components [16].

In this study, characterization using PXRD revealed the formation of a new solid phase different from the diffractograms of the original components. These results are in agreement with those reported previously [17]. Differences in profiles of diffractograms were revealed by the emergence of new diffraction peaks and the loss of specific diffraction peaks from the constituents. These results suggest formation of CQINA.

The shape and size of crystals affect physicochemical characteristics of a solid material such as melting point, solubility, and PXRD profile. The results of SEM indicate that CQINA has a different crystal shape and size when compared to those of its constituents. These results suggest the formation of a co-crystal with new crystal morphology and physicochemical characteristics.

Co-crystals are formed through the interaction between two or more compounds via hydrogen bonds [12]. In this study, the hydrogen bonds formed in CQINA were revealed by FTIR spectra. The wavelength of OH -group in quercetin shifted from 3413.52 to 3215.52 cm^{-1} in CQINA spectrum. The C = O group shift in quercetin also appeared from 1667.62 to 1688.47 cm^{-1} in CQINA spectrum. However, the NH_2 group in INA spectrum which peaked at 3371.33 and 3186.38 cm^{-1} was not observed in CQINA spectrum. It is likely that the NH_2 group was involved in hydrogen bonds formation with the OH group on ring A of quercetin, thus becoming the NH group that peaked at 3416.39 cm^{-1} in the CQINA spectrum.

A shifting and loss of peaks in NH_2 groups showed that the hydrogen bonds interaction between OH groups in quercetin and NH_2 groups in INA formed CQINA.

Dissolution of drugs with very low water solubility is usually the rate-limiting step in the absorption of such drugs in the GIT. Therefore the dissolution of compounds that are sparingly soluble in water can be used to predict their bioavailability. The dissolution of CQINA was slightly increased, relative to those of quercetin and physical mixture of quercetin and INA ($p < 0.05$). Changes in crystal physicochemical properties caused by quercetin interaction with INA produced CQINA with higher dissolution than that of pure quercetin.

CONCLUSION

These results indicate that quercetin and INA (1:1 molar ratio) subjected to solvent evaporation result in the formation of a co-crystal (CQINA) with crystal habits and physicochemical characteristics different from those if its constituents. Modification of quercetin crystals in CQINA increases its *in-vitro* dissolution, thus making it a potential pharmaceutical agent.

DECLARATIONS

Acknowledgement

This work was supported with grants from the Directorate General of Resources for Science, Technology and Higher Education, Ministry of Research, Technology and Higher Education (KEMENRISTEK DIKTI) with scheme of scholarship funding of PhD program at Airlangga University, Surabaya, Indonesia. The authors would like to acknowledge with thanks the Faculty of Pharmacy, Airlangga University and Jember University for assistance with laboratory facilities.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Duenas M, Gonzalez-Manzano S, Gonzalez-Paramas A, and Santos-Buelga C. Antioxidant evaluation of O-methylated metabolites of catechin, epicatechin and quercetin. J Pharm Biomed Anal. 2010; 51: 443-449.*
2. *Nabavi S F, Russo G L, Daglia M, Nabavi S M. Role of quercetin as an alternative for obesity treatment: You are what you eat! Food Chem. 2015; 179: 305-310.*
3. *Kumari A, Yadav S K, Pakade Y B, Singh B, Yadav S C. Development of biodegradable nanoparticles for delivery of quercetin. Colloids Surf B Biointerfaces. 2010; 80: 184-192.*
4. *Anandam S, Selvamuthukumar S. Fabrication of cyclodextrin nanosponges for quercetin delivery: Physicochemical characterization, photostability, and antioxidant effects. J Mater Sci. 2014; 49: 8140-8153.*
5. *Rattanachaikunsopon P, Phumkhachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of Psidium guajava. J Med Plant Res. 2010; 4: 393-396.*
6. *Harwood M, Danielewska B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A Critical Review of the Data Related to the Safety of Quercetin and Lack of Evidence of In Vivo Toxicity, Including Lack of Genotoxic/ Carcinogenic Properties. Food Chem Toxicol. 2007; 45 (11): 2179 - 2205.*
7. *Knab AM, Shanely RA, Henson DA, Jin F, Heinz SA, Austin MD, Nieman DC. Influence of Quercetin Supplementation on Disease Risk Factors in Community-Dwelling Adults. J Am Diet Assoc. 2011; 111(4): 542 – 549.*
8. *Wang X, Ouyang YY, Liu J, Zhao G. Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. Br J Nutr. 2016; 111: 1-11.*
9. *Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm. 2011; 404: 231-237.*
10. *Smith AJ, Kavuru P, Wojtas L, Zaworotko MJ, Shytle RD. Cocrystal of Quercetin with Improved Solubility and Oral Bioavailability. Mol. Pharmaceut 2011; 8(5): 1867 – 1876.*
11. *Krisnalah YSR. Pharmaceutical Technologies for Enhancing Oral Bioavailabilities of Poorly Soluble Drugs. J Bioequiv Availab. 2010; 2(2): 28-36.*
12. *Santos OMM, Reis MED, Jacon JT, Lino MES, Simoes JS, Doriguetto AC. 2014. Polimorphism: An Evaluation of the Potential Risk to the Quality of Drug Products from the Farmacia Popular Rede Propria. Braz J Pharm Sci. 2014; 50 (1): 1-24.*
13. *Setyawan D, Sari R, Yusuf H, Primaharinastiti R. Preparation and Characterization of Artesunate-Nicotinamide Cocrystal by Solvent Evaporation and Slurry Method. Asian J Pharm Clin Res. 2014, 7: 62 – 65.*
14. *Setyawan D, Fadhil AA, Juwita D, Yusuf H, Sari R. Enhancement of Solubility and Dissolution Rate of Quercetin with Solid Dispersion System Formation using Hydroxypropyl Methyl Cellulose Matrix. Thai J Pharm Sci. 2017, 41(3): 112 – 116.*
15. *Yamashita H, Hirakura Y, Yuda M, Teramura T, Terada K. Detection of Cocrystal Formation Based on Binary Phase Diagram Using Thermal Analysis. Pharm Res. 2013; 30(1): 70-80.*
16. *Schultheiss N, Newman A. Pharmaceutical Cocrystals and Their Physicochemical Properties. Cryst Growth Des. 2009; 9(6): 2950 –2967.*
17. *Setyawan D, Jovita RO, Iqbal M, Paramanandana A, Yusuf H, Lestari ML. Co-crystalization of quercetin and malonic acid using solvent-drop grinding method. Trop J Pharm Res. 2018; 17(6): 997-1002.*