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RESEARCH ARTICLE

Improving Dissolution Rate of Piperine by Multicomponent Crystal Formation with Saccharin

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ABSTRACT:
Piperine, a secondary metabolite of *Piper nigrum* L., has been known for its pharmacological activities. However, the use of piperine is still limited due to the low solubility in water. The aim of this study was to improve the physicochemical properties of piperine by preparing into multicomponent crystal (MC) using saccharin by solvent evaporation method, and ethanol was used as the solvent. The intact materials and MC were characterized by several solid-state instruments. The amount of dissolved piperine was determined by High Performance of Liquid Chromatography (HPLC) using acetonitrile: water (90:10) as the mobile phase. Both morphology of intact piperine and MC showed irregular crystals. The diffractogram showed that MC had new and specific peaks at 2Θ: 12.91, 15.04, 19.54 and 22.40. The thermogram presented melting point for intact piperine, saccharin and MC which were 132.81°C, 230.02°C, 197.09°C, respectively. The infra-red spectrum showed no significant shift of MC which indicated no chemical interaction between piperine and saccharin. The dissolution study pointed higher amount of piperine dissolved in 0.1 N HCl with addition of 0.5% sodium lauryl sulphate. The dissolution piperine in MC after 60 minutes was 81.29±5.91%, while intact piperine was 44.78±1.89%. In conclusion, the formation of multicomponent crystal of piperine-saccharin was able to increase the dissolution of piperine.

KEYWORDS: Piperine, Saccharin, Multicomponent Crystal, Solvent Evaporation, Dissolution.

INTRODUCTION:
Pepper or *Piper nigrum* L., known as "the King of Spices", is a family plant Piperaceae which grows in the tropic land that has sufficient rainfall throughout the year and one of the spices that is very important in world trade in the past, present and future. Indonesia is one of the largest pepper producers in Sumatera island, particularly Lampung and Bangka Island[1]. Pepper contains volatile oil about 0.4 - 7%, which contributes to the aroma and spicy taste of pepper. The most active compound in pepper is piperine which is about 2 - 9% from pepper plants[2,3]. Ethnopharmacologically, pepper has been used by Chinese and Indian people to overcome various kinds of diseases; pain, fever, influenza, migraine headaches, increase appetite and facilitate blood flow[4–7].

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In addition, piperine has also been used scientifically to enhance the bioavailability of drugs[8,9]. However, piperine belongs class II based on Biopharmaceutical Classification System (BCS) due to the low solubility in water[10].

Previous studies have reported some modification and improvement in physicochemical properties and pharmacological activity of piperine. The formation of a piperine inclusion complex with beta cyclodextrin[11], formation of piperine solid lipid nanoparticles with polysorbate-80 polymers[12], transdermal delivery systems of piperine preparations[13], formation of piperine nanoparticles with PEGylated-poly (lactic-co-glycolate acid) (PLGA)[14], piperine liposome formulation[15] and the manufacture of a solid dispersion system using the hot melt extrusion technique with several polymers[16] have been reported to increase the solubility and dissolution rate of piperine. One of attractive techniques to modify the solid-state properties in order to enhance solubility and dissolution rate is formation of multicomponent crystal[17]. Multicomponent crystal of pharmaceutical

materials consists of co-crystal, salt, hydrate and solvate [18]. Modification of physicochemical properties of active pharmaceutical ingredient (API) via crystal engineering becomes a popular approach due to the ability to improve solubility, dissolution rate, compressibility, physical and chemical stability and pharmacological effectiveness[19–23].

In this study, piperine was modified to form a multicomponent crystal using saccharin in order to increase the dissolution rate. The formation of multicomponent was characterized using foremost solid state instruments including scanning electron microscopy (SEM), powder X-ray diffraction, differential scanning calorimetry (DSC) and Fourier Transform Infra-red (FT IR) spectroscopy. The amount of piperine was determined by High Performance Liquid Chromatography (HPLC) apparatus.

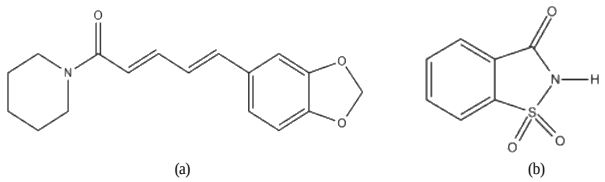

Figure 1. Molecular structure of (a) Piperine, (b) Saccharin

MATERIAL AND METHODS:

Materials:

Piper nigrum L. (identified at Department of Biology, Andalas Univeristy), saccharin (Merck, Germany), ethanol p.a EMSURE® (Merck, Germany), KOH and HCl (Merck, Germany), ethyl acetate (Merck, Germany), Natrium Lauryl Sulphate (Merck, Germany).

Methods:

Isolation of piperine:

The isolation of piperine from *Piper nigrum* L. was done by soxhletation method. About 300 g *Piper nigrum* L. was added to 900 ml of methanol in a Soxhlet extractor for approximately 6 hours. The soxhlet extract was then evaporated using a rotary evaporator (Buchi, Germany) until one of third solvent remained. The sample was then added to 10% KOH and kept for 24 hours to induce the crystal formation. The yellow crystal was then filtered and recrystallized using ethyl acetate. The piperine crystal was then kept in a desiccator.

Preparation of multicomponent crystal (MC):

The equimolar amount of piperine and saccharin (1:1) was dissolved in ethanol and stirred using magnetic stirrer. The solution was then evaporated at room temperature for about 72 hours and the crystal was then kept in a desiccator for characterization.

Scanning Electron Microscopy (SEM) analysis:

The morphology of intact piperine, intact saccharin and the multicomponent crystal was analyzed using a SEM device (HITACHI type S-3400N, Japan) by placing a small amount of sample on the sample holder. Prior to analyze procedure, the SEM device was set as follow: current 12 mA and voltage 10 kV.

Powder X-ray Diffraction (PXRD) analysis:

The PXRD analysis for each sample was conducted using a PANanalytical PW 30/40 X-ray diffractometer (Rigaku, the Netherlands). The diffractometer was set using Kα filter, voltage 45 kV, current 40 mA and target metal Cu. The analysis was recorded 2Θ from 5° to 50°.

Differential Scanning Calorimetry (DSC) analysis:

Approximately 5 mg of each sample was put in an aluminum pan for thermal analysis using a differential scanning calorimetry apparatus (SETARAM Type EVO-131, France) that has been calibrated using Indium prior to measurement. The DSC instrument was set from 40 to 250°C and the heat flow 10 °C/minute.

Fourier Transform Infra-Red (FT IR) analysis:

The FTIR spectra of each sample was generated by a spectrophotometer (Perkin Elmer FT-IR, the USA). The sample was mixed potassium bromide in weight ratio of 1:100. Then, the mixture was compressed into the pellet. The absorption of samples was recorded at wavenumber 4000 – 600 cm-1.

Dissolution study:

Dissolution profiles of intact piperine and multicomponent crystal were determined by a dissolution tester (Copley Scientific NE4-COPD, UK) using paddle type 2. Dissolution apparatus was set at 50 rpm speed, 37 ± 0.5°C temperature in medium of 900 ml 0.1N HCl with addition of 0.5% Sodium Lauryl Sulfate. The solution was sampled at 5, 10, 15, 30, 45 and 60 minutes. The amount of piperine dissolved was measured using HPLC (Hitachi, Japan) using acetonitrile: water (90: 10) as mobile phase at λ 340.2 nm.

RESULTS AND DISCUSSION:

Scanning Electron Microscopy (SEM) analysis:

Piperine is a major secondary metabolite which is produced by Piperaceae family including *Piper nigrum* L. In this study, piperine crystal which was isolated from *Piper nigrum* L. shows rod crystal as seen in Figure 2a. In addition, saccharin as the co-former depicts irregular crystal (see Fig. 2b). Multicomponent crystal which prepared by solvent evaporation shows polyhedral shape, as shown in Fig. 2c.

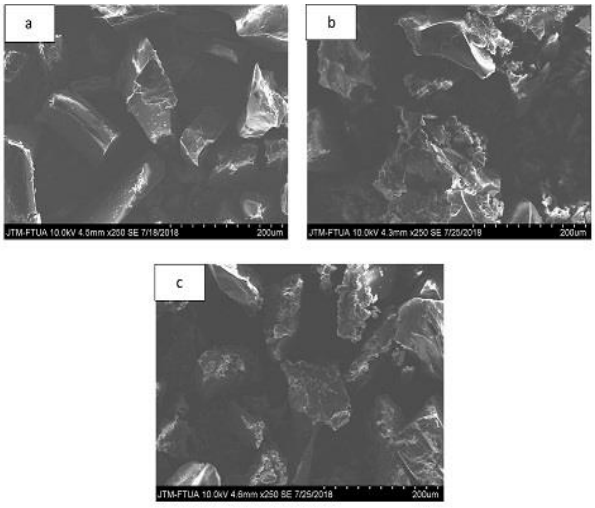

Figure 2. Photomicrograph of (a) Piperine, (b) Saccharin, (c) Multicomponent crystal.

Powder X-ray diffraction analysis:

PXRD analysis is one of the reliable methods to determine the crystalline phase of a solid material, the degree of crystallinity and phase transition. Solid state interaction between two solid drug compounds, which includes the formation of multicomponent crystalline phases, amorphization, solid solutions and highly suitable eutectic mixtures is characterized by the PXRD technique. If the solid-state interaction results in a change in the PXRD pattern, this indicates a new multicomponent crystal phase is formed[24,25].

The PXRD result piperine, saccharine and multicomponent crystal patterns are presented in Figure 3. The piperine and saccharin diffractogram pattern shows a solid phase with a high degree of crystallinity with a typical peak. The pattern of multicomponent crystal piperine-saccharin shows a unique pattern, which is indicated by new peak 2Θ at 12.91, 15.04, 19.54 and 22.40.

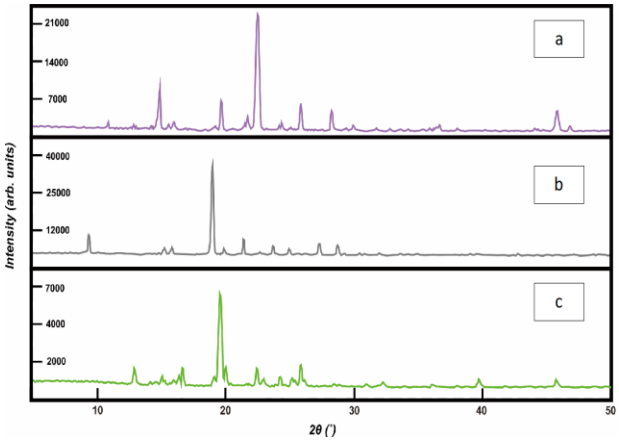

Figure 3. The diffractogram of (a) Piperine, (b) Saccharin and (c) Multicomponent crystal.

Differential Scanning Calorimetry (DSC) analysis:

The DSC thermal analysis was applied to characterize the solid phase thermodynamic properties. Thermal analysis is also often used routinely to screen for multicomponent crystal phase formation[22]. Piperine shows a sharp endothermic peak at 132.8°C, which is the melting point of piperine. The DSC thermogram of saccharin shows also one endothermic peak, at 230.02°C respectively. MC has two endothermic peaks at 110.56 °C and 197.09°C which differ from the two forming components. The endothermic peak at 110.56°C was likely due to the dehydration process of water molecules (hydrate). While the endothermic peak at 197.09°C is the melting point of the crystalline phase of the multicomponent piperine - saccharin, as seen in Figure 4.

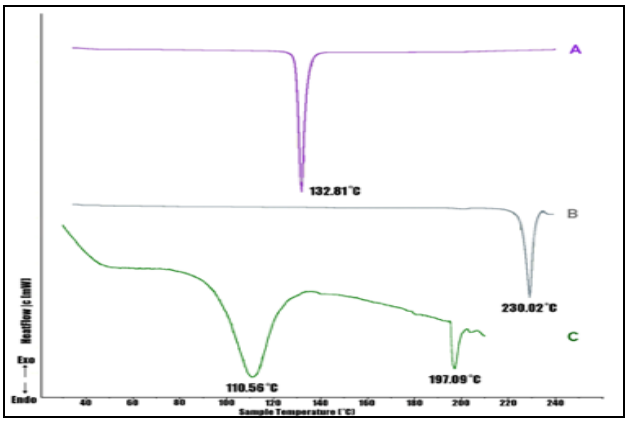

Figure 4. Thermogram of (a) Piperine, (b) Saccharin and (c) Multicomponent crystal.

Fourier Transform Infra-red (FT-IR) spectroscopy analysis:

FT-IR spectroscopy analysis is useful for investigation of intermolecular interactions between two solid components. The presence of transmission band shifts in the FT-IR spectrum can be used to explain the formation of interactions between the two solid components (salt or co-crystal). Spectra of piperine, saccharin and multicomponent crystal were shown in Figure 5. The FT-IR spectra of multicomponent crystal described band change into stronger and broader at 3084.34; 2935.93; 2695.55; 1715.08 and 1440.36 cm^{-1}, respectively. This was estimated due to hydrogen donor (N–H) and acceptors (S=O and C=O) from saccharin bound with C=O and C-H from piperine. Furthermore, this bond is thought to be interaction between N–H.O=C and C–H.O=S, as described in interaction of saccharin with heterocyclic amines[26].

Intermolecular interactions between piperine and saccharin can be predicted based on the pKa rule. Salt type of multicomponent crystal formation is possible because of differences in pKa between piperine and saccharin. Piperine is a weak base with pKa = 13.2[27], while saccharin is weak acid with pKa = 1.6[28]. If pKa

differences between the two components is greater than 3, it allows the formation of salt type multicomponent crystal. Saccharin molecules act as donors of protons in intra-molecular interactions[21,29–31].

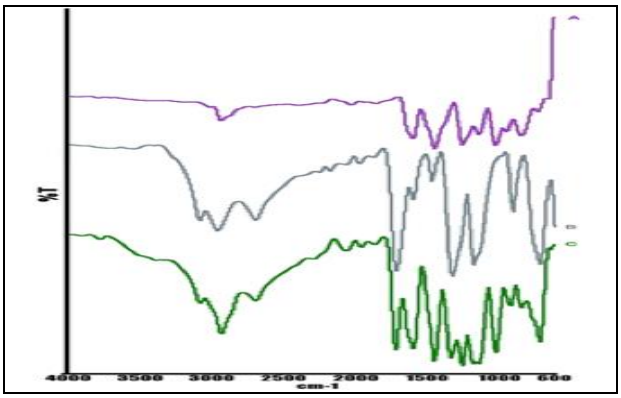

Figure 5. Infra-red spectrum of (a) Piperine, (b) Saccharin and (c) Multicomponent crystal.

Dissolution test:

Dissolution rate is an important part in developing good quality of solid dosage form [32]. In this study, we prepared and characterized multicomponent crystal of piperine-saccharin phase to in order to be able to increase the dissolution. The dissolution profile of piperine and multicomponent crystal is shown in Figure 6. As can be seen in Fig. 6, the amount of piperine dissolved in multicomponent crystal was greater compared to intact piperine. This enhancement was likely due to several factors. Firstly, the multicomponent crystal which likely to form salt type has higher affinity in water that would easily dissociate into anion and cation. Moreover, the decrease of crystallinity in multicomponent crystal contributed to less intermolecular interaction in crystal lattice. The lower interaction molecular in crystal lattice, the lower energy of the crystal, thus the amount of enthalpy decreased as the lower melting point. This result was also shown in other studies [33–37].

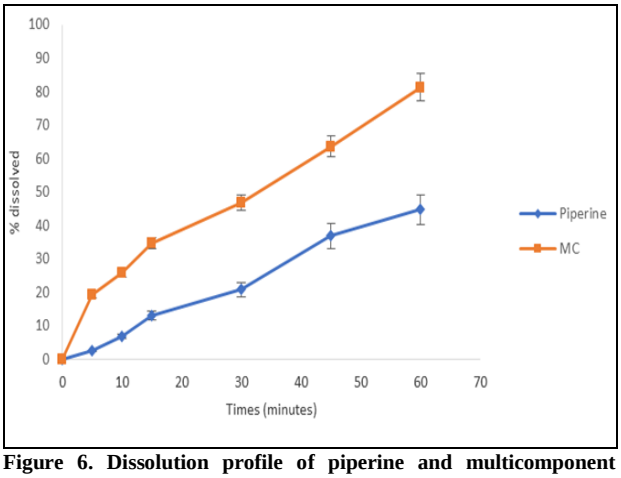

Figure 6. Dissolution profile of piperine and multicomponent crystal (MC).

CONCLUSION:

This study presented a multicomponent crystal of piperin and saccharin which had been prepared by solvent evaporation method. Based on psychochemical characterization, piperine-saccharin has successfully formed into salt-type multicomponent crystal. The multicomponent crystal showed the enhancement in dissolution rate which almost two times compared to intact piperine.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Rukmana R. Usaha Tani Lada Perdu. Indonesia: Kanisius; 2003.
2. Agarwal OP. *Chemistry of Organic Natural Products*. Meerut, India: Goel Publishing House; 2010.
3. Thakur R, Meena AK, Dixit AK, Joshi S. A Review on Different Sources of Piper nigrum L. Adulterants. *Res J Pharm Technol*. 2018;11(9):4173-4178.
4. Gorgani L, Mohammadi M, Najafpour GD, Nikzad M. Piperine—The Bioactive Compound of Black Pepper: From Isolation to Medicinal Formulations. *Compr Rev Food Sci Food Saf*. 2017;26(2):162-168.
5. Ghiware NB, Nesari TM. Antipyretic Activity of Piper nigrum and Nyctanthes arbor-tristis in Different Dosage Forms. *Res J Pharm Technol*. 2010;3(1):157-160.
6. Prabhu A, Chembili V, Kandal T, Punchappady-Devasya R. Piper nigrum seeds inhibit biofilm formation in Pseudomonas aeruginosa strains. *Res J Pharm Technol*. 2017;10(11):3894-3898.
7. Nandakumar S, Kumar MGS, Bini B, Krishnan GG. Antimicrobial activity of selected medicinal plants against oral microflora. *Res J Pharm Technol*. 2016;9(12):2271.
8. Mokkapati A, Nagumantri RK, Pydi CB, Chintala R, Rentala S. Docking Studies of Piperine-Vitamin a Conjugate to Study the Increase in Bioavailability of Vitamin A. *Res J Pharm Technol*. 2017;10(7):2189-2193.
9. Devi UP, Surendran S, Babu M, Joseph J. Beneficial Interaction of Piperine with Sodium Valproate against maximal Electroshock induced Seizures in Mice. *Res J Pharm Technol*. 2017; 10(11):3967-3968.
10. Sharma A, Jain CP, Ashawat MS. Biopharmaceutics classification system (BCS) and biowaivers: role in drug product design. *Res J Pharm Technol*. 2008;1(3):144-151.
11. Ezawa T, Inoue Y, Murata I, Takao K, Sugita Y KI. Characterization of the Dissolution Behavior of Piperine/ Cyclodextrins Inclusion Complexes. *AAPS Pharm Sci Tech*. 2018;19(2):923-933.
12. Yusuf M, Khan M, Khan RA, Ahmed B. Preparation, characterization, in vivo and biochemical evaluation of brain targeted Piperine solid lipid nanoparticles in an experimentally induced Alzheimer's disease model. *J Drug Target*. 2013;21(3):300-311.
13. Alomrani AH, Alhazza FI, AlGhamdi KM, El Maghraby GM. Effect of neat and binary vehicle systems on the solubility and cutaneous delivery of piperine. *Saudi Pharm J*. 2018;26(2):162-168.
14. Pachauri M, Gupta ED, Ghosh PC. Piperine loaded PEG-PLGA nanoparticles: Preparation, characterization and targeted delivery for adjuvant breast cancer chemotherapy. *J Drug Deliv Sci*

Technol. 2015;29:269-282.
15. Priprem A, Chonpathompikunlert P, Sutthiparinyanont S, Wattanathorn J. Antidepressant and cognitive activities of intranasal piperine-encapsulated liposomes. *Adv Biosci Biotechnol*. 2011;2(02):108-116.
16. Ashour EA, Majumdar S, Alsheteli A, et al. Hot melt extrusion as an approach to improve solubility, permeability and oral absorption of a psychoactive natural product, piperine. *J Pharm Pharmacol*. 2016;68(8):989-998.
17. Weyna DR, Cheney ML, Shan N, et al. Improving solubility and pharmacokinetics of meloxicam via multiple-component crystal formation. *Mol Pharm*. 2012;9(7):2094-2102.
18. Grothe E, Meekes H, Vlieg E, Ter Horst JH, De Gelder R. Solvates, Salts, and Cocrystals: A Proposal for a Feasible Classification System. *Cryst Growth Des*. 2016;16(6):3237-3243.
19. Setyawan D, Permata SA, Zainul A, Maria LAD, Lestari. Improvement in vitro Dissolution Rate of Quercetin Using Cocrystallization of Quercetin-Malonic Acid. *Indones J Chem*. 2018;18(3):531-536.
20. Hiendrawan S, Veriansyah B, Widjojokusumo E, Soewandhi SN, Wikarsa S, Tjandrawinata RR. Physicochemical and mechanical properties of paracetamol cocrystal with 5-nitroisophthalic acid. *Int J Pharm*. 2016;497(1-2):106-113.
21. Putra OD, Umeda D, Nugraha YP, Nango K, Yonemochi E, Uekusa H. Simultaneous Improvement of Epalrestat Photostability and Solubility via Cocrystallization: A Case Study. *Cryst Growth Des*. 2018;18(1):373-379.
22. Yuliandra Y, Zaini E, Syofyan S, et al. Cocrystal of ibuprofen–nicotinamide: Solid-state characterization and in vivo analgesic activity evaluation. *Sci Pharm*. 2018;86(2):23-34.
23. Samineni R, Chimakurthy J, Sumalatha K, et al. Co-Crystals: A Review of Recent Trends in Co Crystallization of BCS Class II Drugs. *Res J Pharm Technol*. 2019;12(7):3117-3124.
24. Zaini E, Sumirtapura YC, Soewandhi SN, Halim A, Uekusa H, Fujii K. Cocrystalline phase transformation of binary mixture of trimethoprim and sulfamethoxazole by slurry technique. *Asian J Pharm Clin Res*. 2010;3(4):26-29.
25. Muddukrishna BS, Bhat K, Shenoy GG. Preparation and solid state characterization of paclitaxel cocrystals. *Res J Pharm Technol*. 2014;7(1):6.
26. Sudhakar P, Kumar SV, Vishweshwar P, Babu JM, Vyas K. Solid state structural studies of saccharin salts with some heterocyclic bases. *CrystEngComm*. 2008;10(8):996-1002.
27. Khajuria A, Zutshi U, Bedi KL. Permeability characteristics of piperine on oral absorption - An active alkaloid from peppers and a bioavailability enhancer. *Indian J Exp Biol*. 1998;36:46-50.
28. Perumalla SR, Pedireddi VR, Sun CC. Design, synthesis, and characterization of new 5-fluorocytosine salts. *Mol Pharm*. 2013;10(6):2462-2466.
29. Cruz-Cabeza AJ. Acid–base crystalline complexes and the pKa rule. *CrystEngComm*. 2012;14(20):6362-6365.
30. Childs SL, Stahly GP, Park A. The salt-cocrystal continuum: The influence of crystal structure on ionization state. *Mol Pharm*. 2007;4(3):323-338.
31. Dwichandra Putra O, Yonemochi E, Uekusa H. Isostructural Multicomponent Gliclazide Crystals with Improved Solubility. *Cryst Growth Des*. 2016;16(11):6568-6573.
32. Suhesti TS, Fudholi A, Martien R, Martono S. Pharmaceutical nanoparticle technologies: An approach to improve drug solubility and dissolution rate of Piroxicam. *Res J Pharm Technol*. 2017;10(4):968.
33. Putra OD, Umeda D, Fujita E, et al. Solubility improvement of benexate through salt formation using artificial sweetener. *Pharmaceutics*. 2018;10(2):64-76.
34. Duggirala NK, Perry ML, Almarsson Ö, Zaworotko MJ. Pharmaceutical cocrystals: Along the path to improved medicines. *Chem Commun*. 2016;52(4):640-655.
35. Serajuddin ATM. Salt formation to improve drug solubility. *Adv Drug Deliv Rev*. 2007. doi:10.1016/j.addr.2007.05.010
36. Ainurofiq A, Mauludin R, Mudhakir D, et al. Improving mechanical properties of desloratadine via multicomponent crystal formation. *Eur J Pharm Sci*. 2018;111:65-72.
37. Nugrahani I, Utami D, Ibrahim S, Nugraha YP, Uekusa H. Zwitterionic cocrystal of diclofenac and L-proline: Structure determination, solubility, kinetics of cocrystallization, and stability study. *Eur J Pharm Sci*. 2018;117:185-176.