Trop J Nat Prod Res, April 2021; 5(4):673-677

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Preparation of Curcumin and Quercetin Multicomponent Crystals Via Solvent-Drop Grinding

Lili Fitriani*, Astika Astika, Uswatul Hasanah, Erizal Zaini

Department of Pharmaceutics, Faculty of Pharmacy, Andalas University, Padang 25163, West Sumatera, Indonesia

ARTICLE INFO

Article history:
Received 22 January 2021
Revised 11 March 2021
Accepted 13 April 2021
Published online 03 May 2021

ABSTRACT

Curcumin, a major component of *Curcuma longa* L. (turmeric), has several pharmacological activities; however, its use in pharmaceuticals is limited by poor water solubility. This work sought to improve the solubility of curcumin by preparing it as multicomponent crystals (MC) with quercetin as the co-former. The optimal mole ratio for preparing the curcumin-quercetin MC was determined from a binary phase diagram of their binary mixtures (BMs) at mole ratios of 0.1:0.9 to 0.9:0.1. This ratio was used to prepare the MC by the solvent-drop grinding method in ethanol. MC were characterized using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and Fourier transform infrared (FT-IR) spectroscopy. A simple eutectic mixture formed in the BM at a 0.7:0.3 mole ratio. PXRD results showed a decrease in the intensity of the diffraction patterns, indicating a decrease in the MC crystallinity. Similarly, the DSC thermogram revealed a reduced curcumin endothermic peak in the MC at 171.74 °C. The FT-IR spectrum showed minimal shift of the MC absorption peak. Following sonication in 40 % ethanol for 30 min, the quantity of dissolved curcumin was determined by high performance liquid chromatography at 422 nm using methanol:distilled water (80:20) as the mobile phase. The solubility of the MC and pure curcumin were 124.28 ± 7.076 mg/100 mL and 93.93 ± 6.656 mg/100 mL, respectively, indicating that the curcumin-quercetin MC were more soluble than pure curcumin.

***Keywords*:** Curcumin, Quercetin, Multicomponent crystals, Eutectic mixture, Solubility.

Introduction

Curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; $C_{21}H_{20}O_6$, MW = 368.4 g/mol) is a yellow-orange, crystalline powder isolated from turmeric (*Curcuma longa* L) (Figure 1). It is a polyphenol and a major secondary metabolite of this plant and has been reported to display remarkable pharmacological activities, including antioxidant, antibacterial, anti-inflammatory, and antiproliferative.[1] Curcumin has also been suggested to have therapeutic benefits in neurodegenerative, cardiovascular, liver diseases and diabetes mellitus.[2] Unfortunately, the clinical use of curcumin is limited by its physicochemical properties, including poor water solubility, instability in alkaline conditions, and low bioavailability.[3] While it is soluble in alcohol and glacial acetic acid, particularly ethanol and acetic acid, it is only soluble in water up to 3.12 mg/L, and is insoluble in cold water and ether. It has a pKa of 9.06 and a melting point of 179–182°C.[5,6]

According to the Biopharmaceutical Classification System (BCS), curcumin is classified as class IV, having low solubility in water and low permeability.[4] Several studies have attempted to improve the solubility and permeability of curcumin using a variety of methods, including preparing curcumin-loaded liposomes[7] and solid dispersions of curcumin with polyvinylpyrrolidone (PVP),[8] co-precipitating curcumin with calcium carbonate,[9] and forming curcumin lipid nanoparticles.[10]

*Corresponding author. E mail: lilifitriani@phar.unand.ac.id
Tel: +6281214320154

Citation: Fitriani L, Astika A, Hasanah U, Zaini E. Preparation of Curcumin and Quercetin Multicomponent Crystals Via Solvent-Drop Grinding. Trop J Nat Prod Res. 2021; 5(4):673-677. doi.org/10.26538/tjnpr/v5i4.14

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Multicomponent crystals (MC), defined as crystals with two or more different molecules in a crystal lattice, have been used to improve the solubility and bioavailability of active compounds. MC can be classified into solvates/hydrates, salts, simple eutectics, and co-crystals. A benefit of MC is the ability to alter the physicochemical properties of a molecule without changing its chemical properties.[11] Several methods have been used to prepare MC, such as solvent-drop grinding, solvent evaporation, solid-state grinding, and slurry.[12–15] Previous work showed that a co-crystal of curcumin could be formed using the solvent-drop grinding method with the addition of several co-formers, including resorcinol and pyrogallol, which increased the solubility of curcumin by 4.72 and 11.76, respectively.[16] Another study enhanced the dissolution rates of curcumin by forming a eutectic mixture with salicylic acid and a co-crystal with hydroxyquinol.[17]

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one; $C_{15}H_{10}O_7$, MW = 302.23 g/mol) is a needle-shaped, yellow crystal (Figure 2). Its pKa values are pKa1 = 7.17, pKa2 = 8.26, pKa3 = 10.13, pKa4 = 12.30, and pKa5 = 13.11. Quercetin is highly soluble in ether and methanol, soluble in ethanol and acetone, and insoluble in water and has a melting point of 316.5 °C.[18] Quercetin was chosen as a co-former to prepare MC with curcumin in this work because it is regarded as 'generally recognized as safe' (GRAS) by the Food and Drug Administration (FDA) and has been used in previous studies.[19,20] Quercetin is a known bioenhancer, acting via inhibition of cytochrome P4-A4 and modulation of P-glycoprotein. Studies have described its bioenhancing activities with ranolazine, valsartan, tamoxifen, and pioglitazone.[21,22,23] A curcumin and quercetin combination in the form of a nano-emulsion has been investigated for the treatment of neurodegenerative diseases.[24]

This study investigated the formation of MC of curcumin with quercetin by the solvent-drop grinding method. MC were identified and characterized using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and Fourier transform infrared (FT-IR) spectroscopy. The solubility of the MC was investigated and compared with pure curcumin.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Materials

Commercial curcumin and quercetin were purchased from Tokyo Chemical Industry (TCI) (Tokyo, Japan). Methanol (HPLC grade) and ethanol (pro analysis) were obtained from Merck (Darmstadt, Germany). All other solvents were analytical grade.

Methods

Preparation of binary phase diagram

Curcumin and quercetin were mixed homogeneously at mole ratios from 0.1:0.9 to 0.9:0.1 to obtain binary mixtures (BMs) which were analyzed via DSC. A phase diagram was constructed by plotting the endothermic peak of the BM against the mole ratios. The eutectic form, indicated by a DSC thermogram with a single melting point, was used to prepare MC by the solvent-drop grinding method as described below.

Preparation of MC by solvent-drop grinding

Curcumin and quercetin MC were prepared at a 0.7:0.3 mole ratio with 10 min continuous grinding while approximately 1 mL ethanol was added dropwise. The prepared MC were stored in a desiccator.

Differential scanning calorimetry analysis

Thermal analysis was conducted using a DSC apparatus (DSC 8+; Shimadzu Corp., Kyoto, Japan) calibrated with indium. Approximately 3–5 mg of pure curcumin, pure quercetin, BM, and MC were placed on an aluminum-covered plate and analyzed over a temperature range of 30–350 °C at a heating rate of 10°C/min.

Powder X-ray diffraction analysis

PXRD analysis was performed at room temperature on a diffractometer (PW 30/40 X-ray diffractometer; Malvern PANalytical, Almelo, Netherlands) under the following specifications: Cu metal targets, Kα filter, 40 kV voltage, and 40 mA current. Analysis was performed for pure curcumin, pure quercetin, and MC over a range of 2 theta 10–40°.

Fourier transform-infrared spectroscopy analysis

The FT-IR spectra of pure curcumin, pure quercetin, and MC were obtained on an FT-IR spectrophotometer (Perkin Elmer, Waltham, MA, USA). Each sample was mixed with potassium bromide at a 1:100 ratio (w/w) and compressed into pellets. The absorption peaks were recorded at wavenumber 4000–600 cm^{-1}.

Optimization and validation of high performance liquid chromatography analysis

A high performance liquid chromatography (HPLC) method to evaluate solubility was optimized and validated for curcumin on a Shimadzu HPLC (LC-20AD with an AUX220 UV-VIS detector). The methanol:water mobile phase was varied between 75–85 % methanol. Curcumin (20 µL) dissolved in ethanol at 1000 ppm was injected into the HPLC at a flow rate of 1 mL/min and detected at 422 nm. The selectivity, accuracy, precision, linearity, limit of detection (LoD), and limit of quantification (LoQ) of this method were assessed as follows.

a. *Selectivity*

Selectivity was measured to obtain the resolution of the chromatogram. The test was performed using 20 µL pure curcumin dissolved in ethanol and injected into the HPLC under the optimum conditions. Resolution was calculated as the difference between the retention time and peak width.

b. *Accuracy*

Accuracy was evaluated using curcumin solutions in ethanol (20 µL) at 6, 10, and 14 ppm, which were injected into the HPLC and run under the optimum conditions. The test was performed in triplicate.

c. *Precision*

Intra- and inter-day precision were assessed over 2 d, with triplicate measurements for each concentration. Curcumin solutions in ethanol (20 µL) at 6, 10, and 14 ppm were injected into the HPLC. Standard deviation (SD) and relative standard deviation (RSD) were calculated for each concentration.

Linearity

Curcumin solutions in ethanol (20 µL) at 2, 4, 6, 8, 10, 12, and 14 ppm were injected into the HPLC, with each concentration measured in triplicate. The calibration curve equation was obtained using linear regression analysis of the concentrations and average area under the curve (AUC).

LoD and LoQ

LoD and LoQ were calculated using the following equations:

$$LoD = \frac{3Sy}{b}$$

$$LoQ = \frac{10Sy}{b}$$

Where Sy is standard deviation of y-intercept and b is the slope of calibration curve

Solubility test

The solubility of pure curcumin and MC was assessed following 30 min sonication (Elmasonic P 120 H; Elma Schmidbauer GmbH, Singen, Germany) in 40% ethanol at 30 °C. Each sample was filtered through Whatman filter paper (0.45 µm) and 20 µL of the filtrate was injected into the HPLC. The solubility of each sample was measured in triplicate.

Statistical analysis

The solubility of curcumin and MC was analyzed using one-way analysis of variance (ANOVA).

Results and Discussion

DSC measures absorbed or emitted energy. A BM of curcumin:quercetin at a mole ratio of 0.7:0.3 generated a single endothermic peak with a eutectic point at 174.9°C (Figure 3). The eutectic point indicates the lowest temperature at which a given composition of a mixture of compounds simultaneously melts.[25] The eutectic melting point of the optimal BM was lower than the melting point of curcumin (176.79°C) and quercetin (342.77°C), indicating a lower lattice energy which results in faster dissolution.[26,27]

Thermal analysis showed a decrease in the melting point of the curcumin MC (171.74°C) (Figure 4). The decreased melting point was likely due to physical interactions between curcumin and quercetin.[28] This lower melting point suggests a lower lattice energy, which increases the solubility and dissolution profile of the MC.[29]

The unique and sharp diffraction peaks in the PXRD patterns showed that both curcumin and quercetin were crystalline solids (Figure 5). Crystalline curcumin showed specific diffractions at 2θ = 8.795, 14.405, 17.245, 18.085, 24.485, 27.195, and 28.905°, while quercetin exhibited diffraction peaks at 2θ = 15.765, 17.135, 24.165, 27.055, and 31.695°. The powder X-ray diffractogram of the MC showed similar diffraction peaks to its components, with no new diffraction peaks observed. Although no new crystalline phase was formed, the MC showed a decrease in the intensity of the diffraction patterns, suggesting a change in the crystallinity of the MC compared to pure curcumin and quercetin. This indicates that the curcumin-quercetin MC formed a conglomerate of two crystal phases in solid form, called a eutectic mixture.[25]

FT-IR spectroscopy has been used to analyze chemical interactions between solid molecules by observing shifts of the transmission bands. The wavenumber of the MC shifted only slightly compared to curcumin and quercetin, indicating no chemical interactions between the two components (Figure 5 and Table 1).

The HPLC method to analyze solubility was validated to demonstrate its suitability and determine its operational parameters, including accuracy, precision, selectivity, linearity, LoD, and LoQ.[30] Detected at 422 nm, curcumin showed linearity between 2–14 ppm, resulting in a

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

linear regression equation for the three calibration curves of y = 119344x – 9227.6, R^2 = 0.9992 (Figure 5). The curcumin chromatograms showed good peak resolutions, indicating high selectivity. The resolution (R) was 2.076, confirming a high degree of peak separation (R > 1.5).[31] The percentage recovery relative to a known quantity of curcumin was used to measure accuracy and was between 98–102 % (Table 2).[30,31] SD and RSD were used to assess precision (Table 3). As RSD values were low (< 2 %), this method showed high precision.[32] The LoD and LoQ were 0.411 µg/mL and 1.370 µg/mL, respectively.

Curcumin is unstable in neutral, e.g., water, and alkaline media, decomposing up to 90 % within 30 min at pH 7.4.[16,33] The solubility studies found the MC to be 1.3 times more soluble than pure curcumin (p < 0.05). This is consistent with the PXRD and DSC results showing a decreased crystallinity and melting point, which contributes to increased solubility.

Table 1: The FT-IR of Curcumin, Quercetin and MC

Functional groups	Wave number (cm^{-1})		
	Curcumin	Quercetin	MC
O-H	3501	3311	3316
C=O	1682	1666	1663
C=C	1505	1610	1507 and 1602

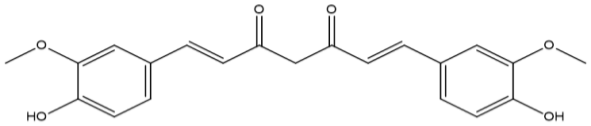

Figure 1: Chemical structure of curcumin

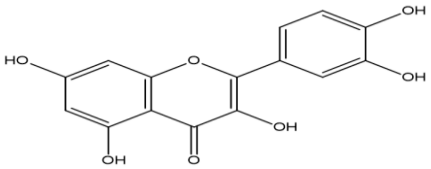

Figure 2: Chemical structure of quercetin

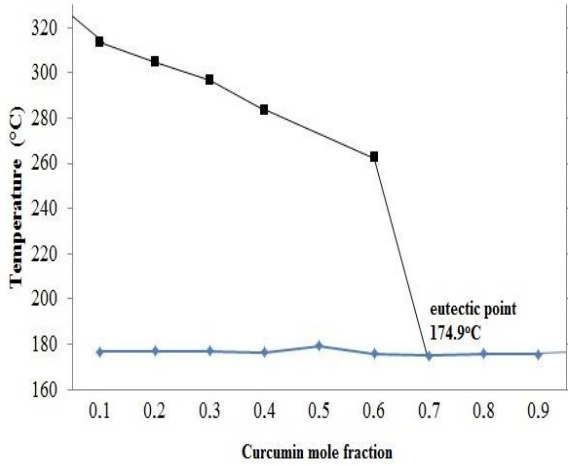

Figure 3: Binary phase diagram of binary mixture (BM) curcumin-quercetin

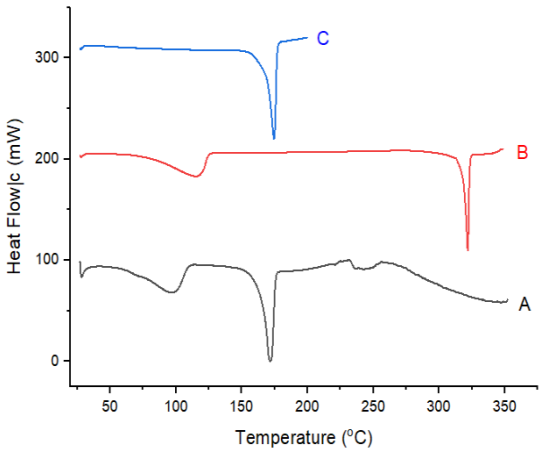

Figure 4: Thermograms of (A) pure curcumin, (B) pure quercetin, (C) MC.

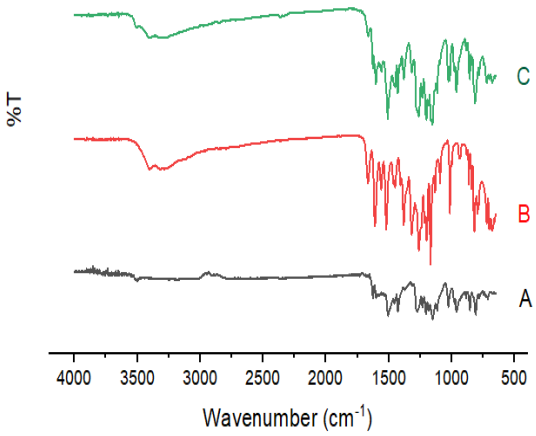

Figure 5: FTIR spectrum of (A) pure curcumin, (B) pure quercetin, and (C) MC.

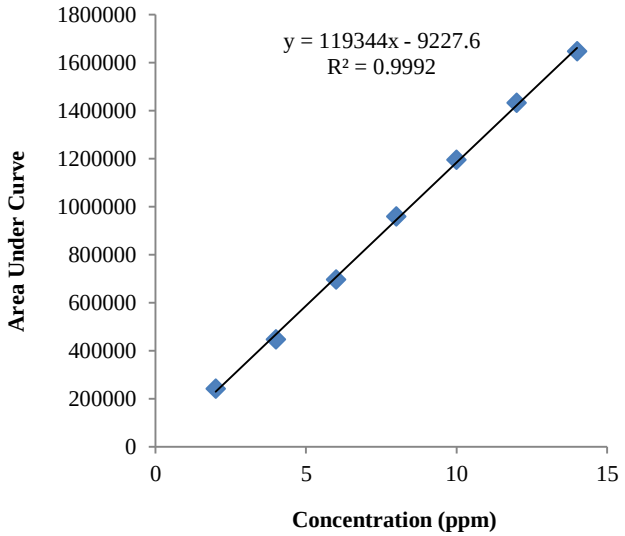

Figure 6: Linearity graph for curcumin for HPLC validation method

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Accuracy of curcumin at different concentrations in ethanol

Concentration (µg/mL)	Recovery (%)		
	1st Replication	2nd Replication	3rd Replication
6.0	98.933	98.200	98.483
10.0	100.610	101.050	100.990
14.0	99.107	99.257	98.950

Table 3: Intra and inter-day of pure curcumin at different concentration in ethanol

Concentration (µg/mL)		RSD (%)	
Intra-day	Inter-day	Intra-day	Inter-day
6	6	0.380	0.551
10	10	0.236	0.681
14	14	0.136	1.267

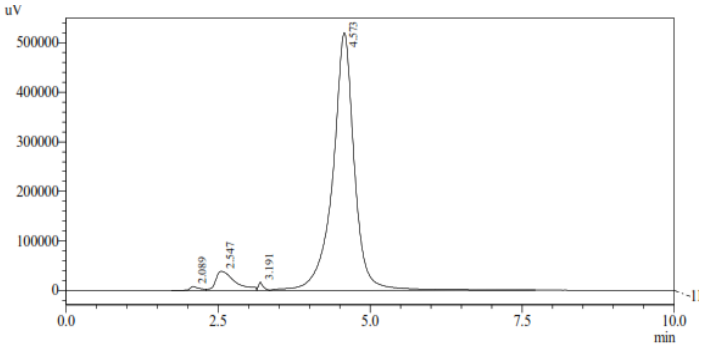

Figure 7: Chromatogram of HPLC analysis of curcumin in methanol:distilled water (80:20)

Conclusion

The characterization and identification of curcumin-quercetin MC using PXRD, DSC, and FT-IR showed the formation of a simple eutectic mixture at a 0:7:0.3 mole ratio. This eutectic mixture has significantly higher solubility than pure curcumin. Preparation of curcumin as MC should be investigated further for use as pharmaceutical solid dosage products.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to thank Andalas University for funded this research grant under scheme Riset Dasar (T/13/UN.16.17/PT.01.03/KO-RD/ 2020) in 2020.

References

1. Ahmad RS, Hussain MB, Sultan MT, Arshad MS, Waheed M, Shariati MA, Plygun S, Hashempur MH. Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review. Evid-Based Compl Altern Med. 2020; 2020:7656919.
2. Rivera-Mancía S, Trujillo J, Chaverri JP. Utility of curcumin for the treatment of diabetes mellitus: evidence from preclinical and clinical studies. J Nutr Intermed Metab. 2018; 14:29-41.
3. Kharat M, Du Z, Zhang G, McClements DJ. Physical and chemical stability of curcumin in aqueous solutions and emulsions: impact of pH, temperature, and molecular environment. J Agric Food Chem. 2017; 65(8):1525-1532.
4. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007; 4(6):807-818.
5. Carolina Alves R, Perosa Fernandes R, Fonseca-Santos B, Damiani Victorelli F, Chorilli M. A critical review of the properties and analytical methods for the determination of curcumin in biological and pharmaceutical matrices. Crit Rev Anal Chem. 2019; 49(2):138-149.
6. Sanphui P and Bolla G. Curcumin, a biological wonder molecule: a crystal engineering point of view. Cryst Growth Des. 2018; 18(9):5690-5711.
7. Chen Y, Wu Q, Zhang Z, Yuan L, Liu X, Zhou L. Preparation of curcumin-loaded liposomes and evaluation of their skin permeation and pharmacodynamics. Molecules. 2012; 17(5):5972-5987.
8. Xu D, Wang S, Jin J, Mei X, Xu S. Dissolution and absorption researches of curcumin in solid dispersions with the polymers PVP. Asian J Pharmacodyn Pharmacokinet. 2006; 6(4):343-349.
9. Zhou H, Wang W, Hu H, Ni X, Ni S, Xu Y, Yang L, Xu D. Co-precipitation of calcium carbonate and curcumin in an ethanol medium as a novel approach for curcumin dissolution enhancement. J Drug Deliv Sci Technol. 2019; 51(1):397-402.
10. Ban C, Jo M, Park YH, Kim JH, Han JY, Lee KW, Kweon DH, Choi YJ. Enhancing the oral bioavailability of curcumin using solid lipid nanoparticles. Food Chem. 2020; 302:125328.
11. 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.
12. Zaini E, Afriyani, Fitriani L, Ismed F, Horikawa A, Uekusa H. Improved solubility and dissolution rates in novel multicomponent crystals of piperine with succinic acid. Sci Pharm. 2020; 88(21):1-12.
13. Zaini E, Sumirtapura YC, Halim A, Fitriani L, Soewandhi SN. Formation and characterization of sulfamethoxazole-trimethoprim cocrystal by milling process. J Appl Pharm Sci. 2017; 7(12):169-173.
14. Zaini E, Fitriani L, Sari RY, Rosaini H, Horikawa A, Uekusa H. Multicomponent crystal of mefenamic acid and n-methyl-d-glucamine: crystal structures and dissolution study. J Pharm Sci. 2019; 108(7):2341-2348.
15. Setyawan D, Jovita RO, Iqbal M, Paramanandana A, Yusuf H, Lestari MLAD. Co-crystalization of quercetin and malonic acid using solvent-drop grinding method. Trop J Pharm Res. 2018; 17(6):997-1002.
16. Sanphui P, Goud NR, Khandavilli UBR, Bhanoth S, Nangia A. New polymorphs of curcumin. Chem Commun. 2011; 47(17):5013-5015.
17. Sathisaran I and Dalvi SV. Crystal engineering of curcumin with salicylic acid and hydroxyquinol as coformers. Cryst Growth Des. 2017; 17(7):3974-3988.
18. Borghetti GS, Carini JP, Honorato SB, Ayala AP, Moreira JCF, Bassani VL. Physicochemical properties and thermal stability of quercetin hydrates in the solid state. Thermochim Acta. 2012; 539:109-114.
19. Andres S, Pevny S, Ziegenhagen R, Bakhiya N, Schäfer B, Hirsch-Ernst KI, Lampen A. Safety aspects of the use of quercetin as a dietary supplement. Mol Nutr Food Res. 2018; 62(1):1-15.

20. Hewlings S and Kalman D. Curcumin: a review of its effects on human health. Foods. 2017; 6(10):92-103.
21. Challa VR, Ravindra Babu P, Challa SR, Johnson B, Maheswari C. Pharmacokinetic interaction study between quercetin and valsartan in rats and in vitro models. Drug Dev Ind Pharm. 2013; 39(6):865-872.
22. Babu PR, Babu KN, Peter PLH, Rajesh K, Babu PJ. Influence of quercetin on the pharmacokinetics of ranolazine in rats and in vitro models. Drug Dev Ind Pharm. 2013; 39(6):873-879.
23. Alexander A, Qureshi A, Kumari L, Vaishnav P, Sharma M, Saraf S, Saraf S. Role of herbal bioactives as a potential bioavailability enhancer for Active Pharmaceutical Ingredients. Fitoter. 2014; 97:1-14.
24. Vaz GR, Clementino A, Bidone J, Villetti MA, Falkembach M, Batista M, Barros P, Sonvico F, Dora C. Curcumin and quercetin-loaded nanoemulsions: physicochemical compatibility study and validation of a simultaneous quantification method. Nanomater. 2020; 10(9):1650.
25. Cherukuvada S and Row TNG. Comprehending the formation of eutectics and cocrystals in terms of design and their structural interrelationships. Cryst Growth Des. 2014; 14(8):4187-4198.
26. Katritzky AR, Jain R, Lomaka A, Petrukhin R, Maran U, Karelson M. Perspective on the relationship between melting points and chemical structure. Cryst Growth Des. 2001; 1(4):261-265.
27. Yuliandra Y, Izadihari R, Rosaini H, Zaini E. Multicomponent crystals of mefenamic acid–tromethamine with improved dissolution rate. J Res Pharm. 2019; 23(6):988-996.
28. Zaini E, Sumirtapura YC, Soewandhi SN. Identifikasi interaksi fisika antara trimetoprim dan sulfametoksazol dengan metode kontak kofler dan reaksi kristalisasi. Maj Farm Indones. 2010; 21(1):32-39.
29. Dwichandra Putra O, Yonemochi E, Uekusa H. Isostructural multicomponent gliclazide crystals with improved solubility. Cryst Growth Des. 2016; 16(11):6568-6573.
30. Singh R. HPLC method development and validation-an overview. J Pharm Educ Res. 2013; 4(1):26-33.
31. Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. J Pharm Biomed Anal. 2018; 147:590-611.
32. Vial J and Jardy A. Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. Anal Chem. 1999; 71(14):2672-2677.
33. Sanphui P, Goud NR, Khandavilli UBR, Nangia A. Fast dissolving curcumin cocrystals. Cryst Growth Des. 2011; 11(9):4135-4145.