JFSP Vol.8, No.3, November 2022, Page: 268-273, pISSN: 2549-9068, eISSN: 2579-4558
Jurnal Farmasi Sains dan Praktis
(JFSP)
http://journal.unimma.ac.id/index.php/pharmacy

VALIDATION OF SPECTROPHOTOMETRIC METHOD TO QUANTIFY QUERCETIN IN THE IN VITRO DISSOLUTION STUDY OF SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS)

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https://doi.org/10.31603/pharmacy.v8i3.6258

Article info:
Submitted : 20-11-2021
Revised : 15-10-2022
Accepted : 06-11-2022

Publisher:
Universitas Muhammadiyah Magelang

ABSTRACT
Quercetin possesses low solubility and decreases oral bioavailability. One way to increase the bioavailability of quercetin is by formulating a self-nanoemulsifying drug delivery system (SNEDDS). In vitro dissolution testing of SNEDDS needs to be carried out using a validated analytical method. This study aims to validate the quercetin analytical method in in vitro dissolution testing. Validation was carried out with two solvents, namely hydrochloric acid buffer pH 1.2 (HCl-1,2) and phosphate buffer pH 6.8 (PO-6.8). It tested some parameters, including linearity, detection limit (LoD), quantification limit (LoQ), accuracy, and precision. The quercetin calibration curve for both solvents has a value of r≥0.999. The LoD at HCl-1.2 and PO-6.8 were 0.26 ppm and 0.27 ppm, respectively. The LoQ of HCl-1.2 and PO-6.8 were 0.86 ppm and 0.91 ppm, respectively. The percentage recovery in both solvents was in the range of 80-110%. The relative standard deviation of the two solvents was less than 7.3%. The quercetin analytical method has been successfully validated as indicated by the results of linearity, detection limit, quantification limit, accuracy, and precision that met the requirements.

Keywords: SNEEDS; Linearity; LoD; LoQ; Accuracy; Precision

1. INTRODUCTION

Currently, there has been a considerable increase in the use of natural products derived from plants in the therapeutic measures to treat diseases. Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one) is a flavonoid that has many potent pharmacological activities. It is contained in abundance in consumable plants in Indonesia, such as onions, apples, various types of berries, nuts, seeds, and various types of leaves and vegetables. Quercetin has been reported to have anticarcinogenic, anti-inflammatory, antiulcer, hypoallergenic, antiviral, and antidiabetic activities (Mukhopadhyay & Prajapati, 2015; Nguyen & Bhattacharya, 2022; Vipin et al., 2020; Wiggers et al., 2022).

However, one of quercetin's drawback in oral delivery is its low solubility. This causes the reduction in its bioavailability and decreases its effectiveness (Tran et al., 2014). Such drawback, thus, serves as a challenge to develop a dosage form that can increase the bioavailability of quercetin in oral delivery.

One way to increase the bioavailability of quercetin is by formulating it into nanoparticles. The nanoparticle formulation is expected to increase absorption, enhance drug stability, and achieve drug delivery to target cells (Rawat et al., 2006). Self-Nano Emulsifying Drug Delivery Systems (SNEDDS) is an applicable type of nanoparticle preparation in the development of quercetin delivery. It is known as a homogeneous mixture of oil, surfactant, cosurfactant, and contains lipophilic active substance. The SNEDDS preparation spontaneously forms a transparent

nanoemulsion with a dispersed particle size of less than 200 nanometers when diluted with water under slow agitation. SNEDDS has a better ability than emulsions and suspensions in increasing the dissolution of lipophilic active substance (Ahmad et al., 2017; Indrati et al., 2020; Tran et al., 2014).

It is necessary to validate the analytical method used in dissolution testing as a way to ensure that the applied method can provide reliable results by testing parameters, such as linearity, detection limit (LoD), quantification limit (LoQ), accuracy, and precision (European Medicines Agency, 1995; Shiyan et al., 2018). On this basis, this study aims to validate the analytical method in the in vitro dissolution test of quercetin SNEDDS. There have been no similar studies that validated the analytical method of quercetin in hydrochloric acid buffer pH 1.2 and phosphate buffer pH 6.8 using UV-Vis spectrophotometry method.

2. METHODS

This research used the following tools: glassware, pH meter (OHAUS ST300), and UV-Vis spectrophotometer (Shimadzu-1800). It also utilized some materials: quercetin (Shaanxi Yuantai Biological Technology Co., Ltd, Shaanxi, China); SNEDDS quercetin, sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH2PO4), sodium chloride (NaCl), hydrochloric acid (HCl) 37% (Merck, Darmstadt, Germany); and aquades (Bratachem, Indonesia).

2.1. Preparation of a 1.2 pH hydrochloric acid buffer solution

First of all, 1.17 g of NaCl was weighed and put into a 100 ml volumetric flask and added with distilled water according to the predetermined mark, which obtained 0.2 M NaCl solution. Then, 1.66 ml of concentrated HCl solution was taken and put into a 100 ml volumetric flask and added with distilled water according to the predetermined mark, which obtained a 0.2 M HCl solution. Following this, 50 ml of 0.2 M NaCl solution and 85 ml of 0.2 M HCl solution were taken and put into a 200 ml volumetric flask. Next, the mixture was added with distilled water up to the limit mark. Finally, the pH was checked and adjusted to reach pH 1.2 (United States Pharmacopeial Convention, 2009).

2.2. Preparation of pH 6.8 phosphate buffer solution

To prepare for pH 6.8 phosphate buffer solution, first of all, 2.72 g of KH_2PO_4 was weighed and put into a 100 ml volumetric flask and added with distilled water according to the mark, which resulted in 0.2 M of KH_2PO_4 solution. Afterwards, 0.8 g of NaOH was weighed and put into a 100 ml volumetric flask and added with distilled water until the limit mark, which resulted in 0.2 M NaOH solution. Following this, 50 ml of 0.2 M $KH_2PO_4$4 solution and 22.4 ml of 0.2 M NaOH solution were taken and put into a 200 ml volumetric flask. Next, the mixture was added with aquadest to the limit mark. Last, the pH was checked and adjusted to reach pH 6.8 (United States Pharmacopeial Convention, 2009).

2.3. Preparation of quercetin calibration curve in pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer

To prepare for quercetin calibration curve in pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer, first of all, 12.5 mg of quercetin was weighed and put it into a 50 mL volumetric flask. In a subsequent step, the mixture was dissolved using each solution of 1.2 pH hydrochloric acid buffer and pH 6.8 phosphate buffer to reach a volume of 50 mL, which obtained a concentration of 250 ppm. The standard curve for quercetin in pH 1.2 hydrochloric acid buffer was made with a series of levels of 3.5 to 13.5 ppm, while the standard curve for quercetin in pH 6.8 phosphate buffer was made with a series of levels of 2.5 to 12.5 ppm. The readings of quercetin absorption were carried out with a maximum wavelength of 366 nm in pH 1.2 hydrochloric acid buffer and 369 nm at pH 6.8 phosphate buffer.

2.4. Linearity

The linearity test was carried out by calculating the correlation coefficient (r) for each solvent used.

2.5. Limit of Detection (LoD) and Limit of Quantification (LoQ)

LoD and LoQ values were calculated based on the value of the standard deviation of the blank (σ) and the slope of the calibration curve (S) as described in the following mathematical equation (1):

$$LoD = \frac{3{,}3\sigma}{S}\,;\, LoQ = \frac{10\sigma}{S} \quad (1)$$

2.6. Accuracy

The accuracy of the analytical method was assessed by calculating the percent recovery. Quercetin standard solution (C_A) was prepared by diluting 250 ppm quercetin stock solution to 6 ppm, 7.5 ppm, and 9 ppm for accuracy calculations in pH 1.2 hydrochloric acid buffer, and 5.2 ppm, 6.5 ppm, and 7.8 ppm for accuracy calculation in pH 6.8 phosphate buffer. Quercetin sample solution (C_B) was prepared by diluting a number of quercetin SNEDDS into a sample solution of 250 ppm, then diluted to 6 ppm, 7.5 ppm, and 9 ppm for accuracy calculations in pH 1.2 hydrochloric acid buffer, and 5.2 ppm, 6.5 ppm, and 7.8 ppm for accuracy calculations in pH 6.8 phosphate buffer. Accuracy solution (C_F) was prepared by taking a number of standard solutions and then adding the same concentration of each sample solution in a volumetric flask. The absorbance of each solution was read using a UV-Vis spectrophotometer at the maximum wavelength of quercetin in each solvent (Muhtadi et al., 2020). The percent recovery was calculated using the following equation (2):

$$\%\text{Percent Recovery} = \frac{\text{Concentration } C_F - \text{ Concentration } C_B}{\text{Concentration } C_A} x100 \quad (2)$$

2.7. Precision

Precision was tested by calculating the relative standard deviation (RSD). Three levels of concentration of sample solution and stock solution were read for absorbance for 3 times and replicated using a UV-Vis spectrophotometer. The RSD value was calculated by the following equation (3):

$$\text{RSD } (\%) = \frac{\text{Standard Deviation}}{\text{Mean Grade}} x100 \quad (3)$$

2.8. Data Analysis

The data were presented in the form of mean ± standard deviation using replication in each test for 3 times.

3. RESULTS AND DISCUSSION

This study used 2 types of solvents: pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer. pH 1.2 hydrochloric acid buffer was used to describe the atmosphere in the stomach, while pH 6.8 phosphate buffer was used to describe the atmosphere in the intestinal tract (Syukri et al., 2018). The graph of the quercetin calibration curve with pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer are presented in **Figure 1**. From these results, it is clear that the linearity values of the two calibration curves have met the requirements of the correlation coefficient value of 0.999. The linearity value describes a measure of the proportionality of the response to the analyte concentration in the sample (Moffat et al., 2011).

The limit of detection and limit of quantification values obtained in this study are presented in **Table 1**. From this result, it is clear that the smallest amount of analyte in the sample that can be detected by the analytical method with pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate

buffer is 0.26 ppm and 0.27 ppm, respectively. The smallest amount of analyte in the sample that can be accurately and precisely quantified and detected by the analytical method of pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer was respectively 0.86 ppm and 0.91 ppm (European Medicines Agency, 1995).

Test results of the accuracy of quercetin can be seen in **Table 2**. The Association of Official Analytical Collaboration (AOAC) determines that the acceptable average value of the percent recovery is between 80-110%. The quercetin accuracy data obtained in this study were within the specified range. Quercetin analysis using pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer obtains the quercetin levels that are close to the actual levels. Accuracy is a validation parameter that describes the closeness of the test results to the true value or to the accepted value. The level of accuracy of an analytical method is expressed by the percent recovery value, which is the percentage of analyte recovered after the sample has passed the testing process. This study used standard addition as the accuracy test method. This method is used when the effect of the matrix on the analyte is unknown or various (AOAC International, 1993).

The quercetin precision test is presented in **Table 3**. The RSD value obtained in this study was at the specified value, which was less than 7.3% in the two solvents used. Parameters of precision or accuracy describe the closeness of the results of the analysis between homogeneous samples and repeated treatments. Based on the requirements by AOAC, precision testing can be carried out with the provision of a minimum of 9 measurements covering 3 concentration series with the number of replications of 3 times in each concentration series (European Medicines Agency, 1995).

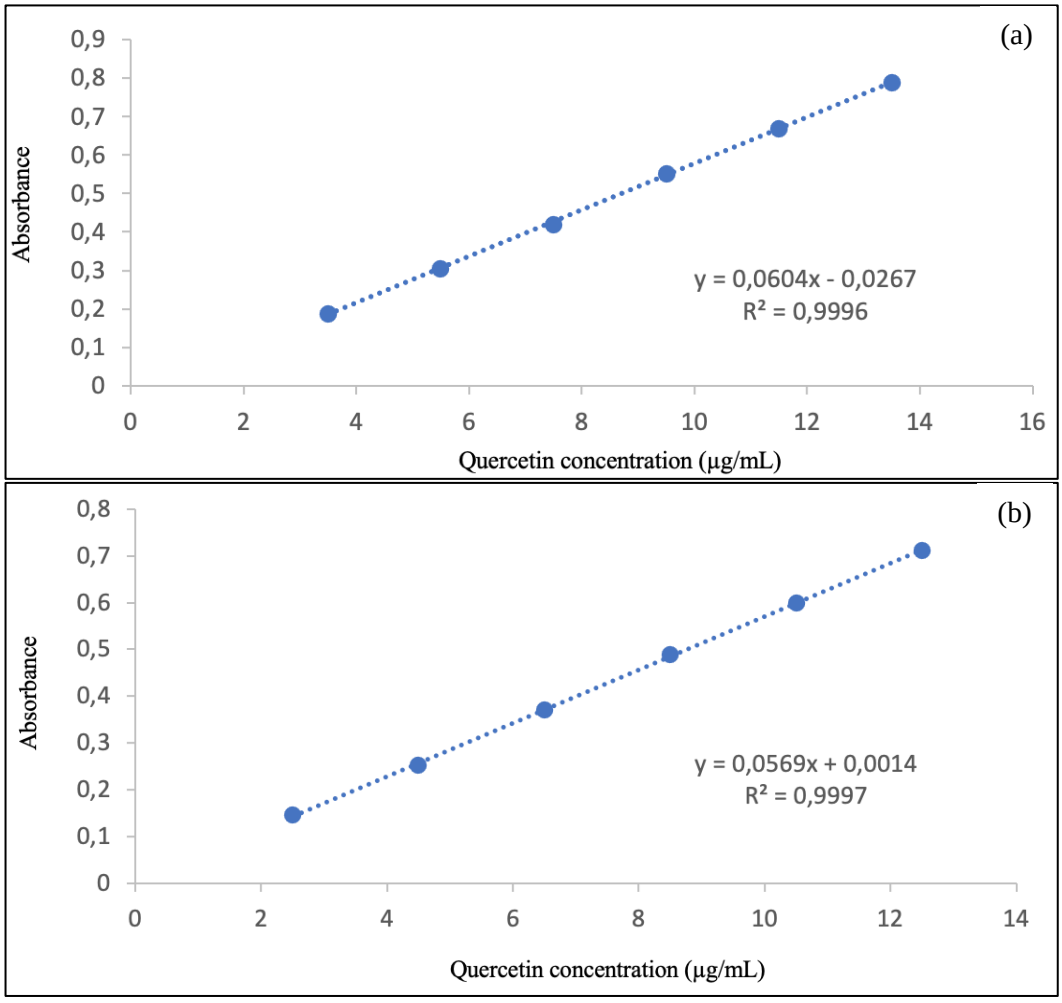

Figure 1. (a) Calibration curve of quercetin with pH 1.2 hydrochloric acid buffer; (b) pH 6.8 phosphate buffer

Table 1. Limits of detection and Limits of quantification of quercetin in pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer.

Solvent	Limit of Detection (ppm)	Limit of Quantification (ppm)
pH 1.2 HCl Buffer	0.26	0.86
pH 6.8 Phosphate Buffer	0.27	0.91

Table 2. Accuracy of quercetin in pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer

Solvent	Theoretical Concentration (ppm)	Average Recovery (%)	Acceptance Criteria (%)
pH 1.2 HCl Buffer	6	101.777±6.664	80-110%
	7.5	101.780±1.350	80-110%
	9	103.908±0.126	80-110%
pH 6.8 Phosphate Buffer	5.2	99.699±1.247	80-110%
	6.5	92.309±10.839	80-110%
	7.8	86.368±1.166	80-110%

Table 3. The precision of quercetin in pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer

Solvent	Theoretical Concentration (ppm)	RSD (%)	Acceptance Criteria (%)
pH 1.2 HCl Buffer	6	1.014	7.3
	7.5	0.268	7.3
	9	1.130	7.3
pH 6.8 Phosphate Buffer	5.2	0.793	7.3
	6.5	0.117	7.3
	7.8	0.196	7.3

4. CONCLUSION

From this research, it is obvious that the analytical method used in the in vitro quercetin SNEDDS dissolution test has met the criteria for linearity, LoD, LoQ, accuracy, and precision. Therefore, the analytical method of quercetin in in vitro dissolution testing using pH 1.2 hydrochloric acid buffer and pH 6.8 phosphate buffer can provide reliable analytical results. However, further research that examines other validation parameters such as specificity in order to further increase the level of confidence in the analytical method used is still needed.

5. ACKNOWLEDGEMENT

The authors would like to thank the 2021 Ministry of Research, Technology and Higher Education Beginner Lecturer Research Grant for funding this research.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose related to the research implementation and research data.

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