Indonesian Journal of Pharmacy

VOL 34 (2) 2023: 272–279 | RESEARCH ARTICLE

Validation of Uv-Vis Spectrophotometric Method to Determine Drug Release of Quercetin Loaded-Nanoemulsion

Vania Santika Putri[1], Arif Nur Ikhsan[2], Ronny Martien[2,3] and Adhyatmika[2,3]*

1. Magister of Pharmacy Science, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia.
2. Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
3. Drug Targeting and Personalized Medicine Research Group, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281

Article Info

Submitted: 20-04-2022
Revised: 26-05-2022
Accepted: 15-08-2022

*Corresponding author
Adhyatmika

Email:
adhyatmika@ugm.ac.id

ABSTRACT

Oral bioavailability of quercetin is limited due to its poor water solubility. The formulation of quercetin into a spontaneously produced nanoemulsion system which increases the oral bioavailability. The quantification of the released quercetin from the nanoemulsion matrix is one of the crucial parameters which requires an adjustment, development and validation of recent analytical methods. A simple validated UV/Vis spectrophotometric method in investigating quercetin released from the nanoemulsion matrix was developed to examine the drug release profile of quercetin loaded-nanoemulsion. Quercetin analysis was administered at a maximum wavelength of 254 nm. The method presented a linearity with a correlation coefficient (r) value of 0.9998 in the range of 4 - 12 µg/mL. The results also unveiled that the procedure is accurate and precise, with recovery (%) in the range of 99.65 - 100.1312 % and RSD (%) as ≤ 2%. The limit of detection (LOD) and limit of quantitation (LOQ) values were revealed to be 4.0535 µg/mL and 13.5118 µg/mL, respectively. The developed method was discovered to be valid to scrutinize quercetin released in nanoemulsion preparations.

Keywords: quercetin, recovery, accuracy, precision

INTRODUCTION

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a natural flavonoid extensively discovered in various fruits and vegetables. It was uncovered to contain several beneficial effects on health, incorporating antibacterial, antiviral, anti-obesity, antioxidant, anticarcinogenic, and anti-inflammatory (Batiha *et al.,* 2020; Pangeni *et al.*, 2017; Yang *et al.*, 2020). Quercetin is considered to be one of the most potent antioxidants among other polyphenols such as curcumin, resveratrol, kaempferol, etc. (Anand David *et al.*, 2016; Madiha *et al.*, 2021). The antioxidant properties of quercetin are due to its free radical scavenging activity which decreases reactive oxygen species (ROS) level and inhibits lipid peroxidation (Abarikwu *et al.*, 2012; Tang et al., 2020; Xu *et al.,* 2019). Quercetin was also considered as a potential anticancer agent since it is able to constrain cell proliferation and induce apoptosis (Nguyen *et al.*, 2017). Its ability is also in inhibiting cell death and discontinuing cancer cell cycle through downregulation of oncogenes, encompassing Bcl-2, Mcl-1, PI3K, and Ras, or by upregulation of suppressor genes such as p21 and p53 (Nguyen *et al.*, 2017; Ranganathan *et al.*, 2015; Spagnuolo *et al.*, 2011). Due to its potential pharmacological effects, quercetin potentially was developed into various pharmaceutical products.

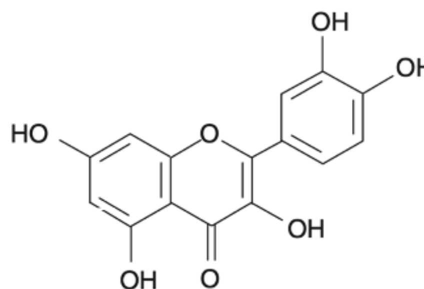

Figure 1. Chemical Structure of Quercetin (Rauf *et al.,* 2018)

Copyright © 2023 by Indonesian Journal of Pharmacy (IJP). The open access articles are distributed under the terms and conditions of Creative Commons Attribution 2.0 Generic License (https://creativecommons.org/licenses/by/2.0/).

However, the oral delivery of quercetin is limited due to the low solubility. Quercetin is classified as a class II compound in the biopharmaceutics classification system (BCS) because of the low solubility and high permeability. It possesses low solubility in water with only approximately 0.01 mg to be dissolved per mL of solvent in 25°C. Meanwhile, in the ethanol, it is 4.0 mg of compound per mL solvent in 37°C (Gao *et al.*, 2011; Priprem *et al.*, 2008). Furthermore, quercetin is chemically unstable in gastrointestinal fluids with an extensive first-pass metabolism and owns rapid elimination after oral administration. Due to the several constraints, quercetin contains low oral bioavailability with only <2% in humans (Cai *et al.,* 2013). Therefore, various approaches to overcome these limitations were employed to enhance oral bioavailability of quercetin.

We were developing a nanoemulsion formulation for quercetin, by optimizing the combination of oleic acid as oil phase, cremophor RH 40 as surfactant, and polyethylene glycol 400 (PEG 400) as co-surfactant to formulate a spontaneously generated oil in water system of nano-sized globules. This nanoemulsion formulation for quercetin requires adjustment, developments, and validation of the latest analytical method to assure that the method administered is fit for purpose. One of the critical parameters in nanoemulsion characterization is the drug release determination from nano-carrier system. The quantization of the released quercetin from the nanoemulsion system is essential to verify and requires to validate to ensure that the quantification of drug levels in the release medium is appropriate. In order to discover the amount of released quercetin from the nanoemulsion system, it is necessary to advance a valid and robust analytical method.

Various analytical methods to unveil drug levels were reported, encompassing UV/Vis spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography/tandem mass spectrometry (LC/MS), and spectrofluorometric (Alva-Ensastegui *et al.*, 2018; Firoozeh *et al.,* 2019; Son *et al.*, 2019). Among the published methods, UV/Vis spectrophotometry method is the most advantageous as it is easy and economically affordable to implement. However, references on the development and validation of analytical methods for quercetin release from nanoemulsion systems by UV/Vis spectrophotometry are tremendously limited. In the current study, the objective of this study is to develop and validate a practical method to investigate the amount of released quercetin from the nanoemulsion system by employing UV/Vis spectrophotometry. The validation of the UV/Vis spectrophotometric method in this study was conducted according to the International Conference on Harmonization (ICH) guidelines, encompassing validation parameters of linearity, precision, accuracy, Limit of Detection (LOD), and Limit of Quantitation (LOQ).

MATERIALS AND METHODS

Chemicals and Reagent

Quercetin was purchased from Octagon Chemicals Limited (China). Potassium phosphate monobasic and sodium dodecyl sulphate (SDS) employed in the study were analytical reagent grades purchased from Merck, while sodium hydroxide (NaOH), ethanol absolute, and deionized water were purchased from Brataco (Indonesia).

Instrumentation

UV/Vis spectrophotometer (Genesys50) instruments with a single-beam detector configuration with a 10 mm pathlength quartz glass cuvette were administered in this study.

Preparation of Solvent

The solvent employed in this study was simulated intestinal fluid (SIF) pH 6.8. The SIF solution pH 6.8 was applied by dissolving potassium phosphate monobasic (3.4 g) and SDS (1.25 g) with 800 mL of deionized water. Then, pH was adjusted to 6.8 ± 0.1 administering 1 N NaOH. Finally, the volume was adjusted to 1000 mL with the deionized water.

Preparation of Standard Stock Solution of Quercetin

Quercetin standard stock solution was applied by accurately weighing 20 mg of standard quercetin, then placing it in a 50.0 ml volumetric flask. The volume was adjusted till mark with ethanol absolute to dissolve quercetin. The solution was obtained 2.0 mL, then transferred into a 10.0 mL volumetric flask. The solution was added with SIF solution pH 6.8, and adjusted the volume till mark to acquire quercetin standard stock solution with final concentration of 80 µg/mL.

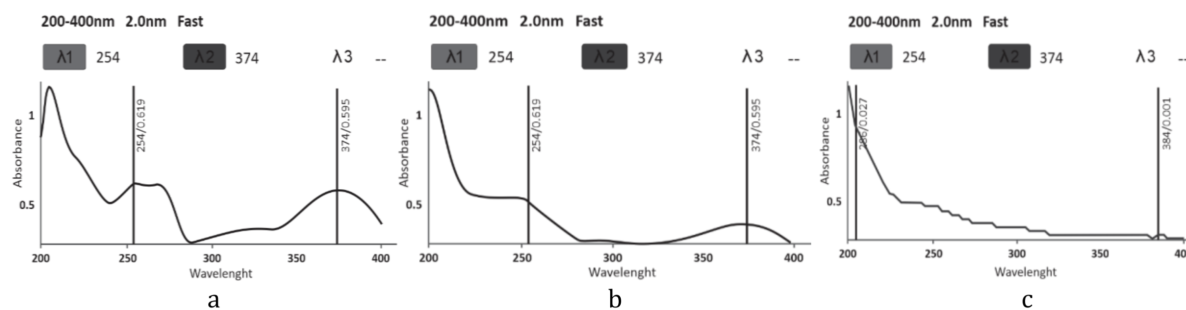

Figure 2. UV/Vis spectrum: (a) quercetin in SIF solution pH 6.8, (b) The released quercetin in SIF solution pH 6.8, (c) nanoemulsion base in SIF solution pH 6.8

Table I. Accuracy Study

Concentration (μg/mL)	% Recovery ± S.D
6	99.65 ± 0.0241
8	100.1312 ± 0.0506
10	99.79 ± 0.0481

Table II. Intraday Precision Study

Concentration (μg/mL)	Mean Concentration (μg/mL)	S.D	% RSD
6	5.96633	0.0328	0.5497
8	8.0052	0.0091	0.1136
10	10.0157	0.0315	0.3145

Table III. Intraday Precision Study

Concentration (μg/mL)	Concentration D.1 (μg/mL)	Concentration D.2 (μg/mL)	Concentration D.3 (μg/mL)	Mean Concentration (μg/mL)	S.D	%RSD
6	5.9213	5.9843	5.9528	5.9528	0.0315	0.5291
8	8.0000	7.9685	8.0787	8.0157	0.0568	0.7084
10	9.9528	9.9685	10.0472	9.9895	0.0506	0.5068

Determination of Maximum Wavelength of Quercetin

The quercetin standard stock solution was further diluted employing SIF solution pH 6.8 to gain a quercetin solution concentration of 4 μg/mL. The absorbance of the solution was scanned at the wavelength range of 200 - 400 nm by administering UV/Vis spectrophotometer with SIF solution pH 6.8 as blank.

Validation of Method Development

The validation of UV/Vis spectrophotometric method was performed based on the International Conference on Harmonization (ICH) guidelines, encompassing the study of linearity, precision, accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Linearity

The quercetin standard stock solutions were diluted employing SIF solution pH 6.8 to acquire a series of solutions with concentrations of 4, 6, 8, 10, and 12 μg/mL. The absorbance of these solutions was measured at a maximum wavelength of quercetin. The standard calibration curve was procures by plotting the absorbance vs the series concentrations of quercetin solutions.

Accuracy

The absorbance of three quercetin solutions with concentrations of 6, 8, and 10 μg/mL was examined administering UV/Vis spectrophotometer at the maximum wavelength of quercetin. The procedure was repeated three times.

The accuracy was determined based on a percentage of recovery (% recovery) parameter.

Precision

The precision of the analytical method in this study was determined based on intraday and interday precision. Intraday precision was performed by evaluating the absorbance of three quercetin solutions with a concentration of 6, 8, and 10 µg/mL three times on the same day. Meanwhile, interday precision was administered on three different days. The percentage of RSD for each procedure was then calculated.

LOD and LOQ

LOD and LOQ was determined based on the data in the form of standard deviation and slope value attained from the calibration curve. LOD and LOQ values were examined by applying the following equation:

LOD = 3.3 x σ/s   LOQ = 10 x σ/s

Where σ is the standard deviation of y-intercepts of regression line, while s is the slope value both obtained from the calibration curve (Figure 2).

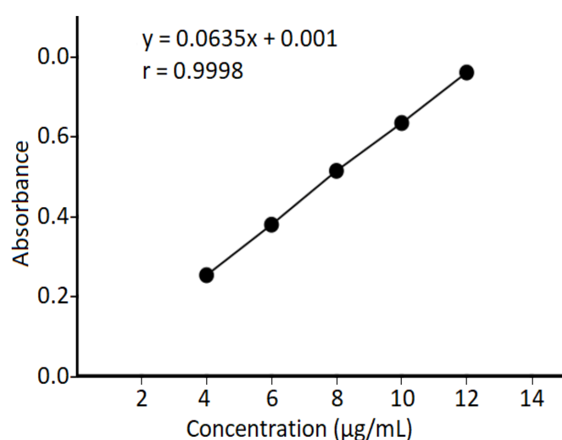

Figure 3. Quercetin calibration curve at the wavelength of 254 nm

Determination of *In Vitro* Drug Release of Quercetin Loaded-Nanoemulsion

Nanoemulsion was conducted by spontaneous emulsification technique by utilizing oleic acid as oil phase, cremophor RH 40 as surfactant, and polyethylene glycol 400 (PEG 400) as co-surfactant with a ratio of 1:6:3. Distilled water was slowly administered with continuous stirring to mark up the total volume of nanoemulsion. Quercetin concentration administered was 0.16 % w/v. *In vitro* drug release from quercetin loaded-nanoemulsion was performed employing the dialysis bag method. Nanoemulsion (1 mL) containing 1.6 mg quercetin was poured into a dialysis bag. The dialysis bag was sealed and placed into 40 mL SIF solution pH 6.8 as the dissolution medium at 37 ± 0.5°C with rotating speed of 100. The sample was withdrawn at predetermined time intervals (0, 5, 15, 45, 60, 90, 120, 180, 240, 300, and 360 min. The quercetin concentration in the samples was assayed utilizing UV/Vis spectrophotometer at the maximum wavelength of quercetin. The percentage of drug release was determined employing the following formula:

$$\% \text{ Drug Release} = \frac{\textit{Released Quercetin}}{\textit{Total Quercetin}} \times 100$$

RESULTS AND DISCUSSIONS

Maximum wavelength of Quercetin:

The maximum wavelength of quercetin in SIF solution pH 6.8 acquired from the absorbance scanning in the range of 200-400 nm is 254 nm (Figure 3). Therefore, the maximum wavelength at 254 nm was determined as a quantification wavelength for further quercetin assay in this study.

Linearity

The correlation coefficient (r) value of ≥ 0.998 implies that the linearity of an analytical procedure is good in the specified concentration range (Yunita *et al.*, 2020). Linearity test results (Figure 3) present a linear calibration curve with a correlation coefficient (r) of 0.9998.

Accuracy

The recovery value (%) in this study met the acceptance criteria according to the Association of Analytical Chemists AOAC since the % recovery value was discovered in the range of 99.65 - 100.1312 % (Table I).

Precision

Both the intraday and interday precision studies revealed that the %RSD value meet the acceptance criteria of ≤ 2% (Table II and III).

LOD and LOQ

The limit of detection and limit of quantification of quercetin measured based on the standard deviation of the y-intercept and the slope of the calibration curve were 4.0535 µg/mL and 13.5118 µg/mL, respectively.

In Vitro Drug Release of Quercetin Loaded-Nanoemulsion

The in vitro release profile uncovered that the release of quercetin from the nanoemulsion matrix increased within 0 to 360 minutes. Quercetin was stable encapsulated in nanoemulsion matrix within 6 h, in which the % release of quercetin from the nanoemulsion system after 6 h merely achieved 83.081%. Quercetin released from the nanoemulsion system after 30 min was 23.604%, while after 60 min was 29.650%. Based on these results, it is indicated the orally administered quercetin nanoemulsion may be absorbed either in the encapsulated form of the nanoemulsion matrix or in its free form. The quercetin loaded-nanoemulsion was completely dissolved in aqueous media of SIF solution pH 6.8 with no aggregates formed. Release profiles of quercetin loaded-nanoemulsion over the period of 6 h in SIF solution pH 6.8 (Figure 4).

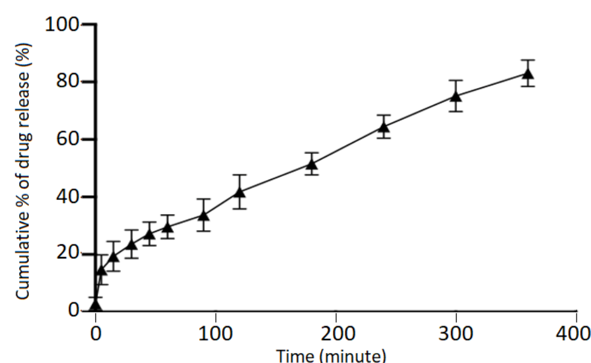

Figure 4. *In vitro* release profile of quercetin loaded-nanoemulsion

The chemical structure of quercetin as a polyphenol occupying two aromatic rings connected by a γ-pyrone ring allows the determination of the compound administering analytical instruments in accordance with UV-Vis detection (Buchweitz *et al*., 2016). When the compound's chemical structure is not compromised by the chemical reaction to another compound in the system, the method should be straightforward. However, this situation may not frequently function in pharmaceutical formulation in which compound combination is commonly required for the benefit of biopharmaceutical profile. Studies on the development and validation of UV/Vis spectrophotometric analysis methods for drug release from nanoparticle matrix particularly for quercetin loaded-nanoemulsions are highly demanded, because of the limitation of references on this topic. In vitro drug release study of quercetin from solid lipid nanoparticles (SLNs) by dialysis method was performed by Niazvand *et al.* (2019) by applying PBS solution (pH 7.4) as the release medium, but the publication did not provide further details on the validation of the proposed analytical method (Niazvand *et al.*, 2019).

In this study, a spontaneously formed nanoemulsion system was advanced based on the principle of the self-nanoemulsifying drug delivery system (SNEDDS) (Changediya *et al.,* 2019). Quercetin as polyphenols was dispersed well in the organic/oil-based solvents, which is not suitable with the water-based system inside the gastro-intestinal tract. Surfactant and co-surfactant were employed to decrease the interfacial tension between the oil-based solvent applied to dissolve quercetin in the preparation to spontaneously formulating nano-sized particles of oil and the polar environment. In performing it, the surfactant and co-surfactant range in the interfacial layer between the two opposing solvents, with the polar head groups to encounter the polar solvent and non-polar tail groups to encounter the oil solvents. The oleic acid administered in our formulation functioned as solvent by containing quercetin, while the cremophor RH40 and PEG400 were infused into the oleic acid in the formulation. There is still a few possible theory and reports suggesting any interaction between either cremophor RH40 or PEG400 to potentially interact with the quercetin.

During emulsification, nano-globules of quercetin-containing oleic acid were generated in the water-based system/buffer, combined by the cremophor RH40/PEG400 layer. In the in vitro study setup, the quercetin was then gradually released from the globules to disperse in the SIF medium and crossed the dialysis membrane without the matrix. Therefore, the quercetin managing to pass the dialysis bag pores were all free quercetin. Therefore, it can be determined without the contamination of any emulsion system employed in the formulation. This approach was corroborated by Hua (2014) in the study on the in vitro dialysis of Loperamide HCl (Hua, 2014).

It is crucial to determine the maximum wavelength of quercetin in SIF solution pH 6.8 in order to enhance sensitivity and minimize errors when repeated measurements are established. The determination of maximum wavelength of quercetin in SIF solution pH 6.8 was calculated in the wavelength range of 200-400 nm by utilizing UV/Vis spectrophotometry. The wavelength scan

result displayed two spectra peaks at 254 nm and 378 nm. The maximum wavelength of quercetin was generated at 254 (absorbance=0,619 nm) for further quercetin assay in this study, because it produced a higher absorbance than 374 nm (absorbance=0.595). Moreover, these results were also corroborated by other studies which unveiled the maximum wavelength of quercetin in various solvents that were adjacent to 254 nm. Other studies revealed that quercetin in phosphate buffered saline (PBS) solution pH 7.4 occupies a maximum wavelength of 256 nm (Niazvand *et al.,* 2019). Patel *et al.* (2020) developed a UV spectrophotometric method and validation for quantitative determination of quercetin in bulk and quercetin loaded microspheres which illustrated the maximum wavelength of quercetin in phosphate buffer pH 7.4 which was 256 nm and the proposed method was highly sensitive, linear, accurate and precise. Furthermore, Patil *et al.* (2012) also formulated a simple and reproducible UV spectrophotometric method for the simultaneous estimation of quercetin in *Cocculus hirsutus* by utilizing the developed solvent n-butanol: water: acetic acid (7:1:1), displaying the maximum wavelength of quercetin at 256.30 nm.

Linearity is the ability of an analytical procedure to provide response within a certain range which is proportional to the analyte's concentration of the sample solution. Linearity of the analytical method in this study was administered between 4 and 12 μg/mL. The correlation coefficient (r) acquired from standard calibration curve was employed as the linearity parameter of the proposed method. The results of the linear regression equation attained was y=0.0635x+0.001, with a correlation coefficient (r) of 0.9998. The correlation coefficient (r) value of ≥0.998 implies satisfactory linearity in the specified concentration range (Yunita *et al.*, 2020).

The accuracy of analytical procedures demonstrates the closeness between the received true value and the measurement value of a sample. The accuracy of the proposed method is determined by the percent recovery. The recovery value (%) was unveiled in the range of 99.65 - 100.1312%, which signifies that the proposed method meets the accuracy requirement. The acceptable criteria of accuracy in accordance with Food and Drug Administration (FDA) guidelines is approximately in the range of 80-120%. UV/Vis spectrophotometric method for quantitation of quercetin in ethanol extract of tamarind leaf proposed by Yunita et al. (2020), revealed recoveries range of 97-103%. Based on the recovery value in this study, it was uncovered that the proposed method meets the accuracy parameters.

The precision of analytical procedures refers to how closely a set of measurements results attained from multiple sampling of the same and homogeneous sample under the specified conditions. Relative standard deviation (RSD) was utilized to represent the values of repeatability (intraday precision) and intermediate precision (interday precision). The acceptance criteria of RSD value based on the International Conference on Harmonization (ICH) is less than or equal to 2%. Both the intraday and interday precision studies demonstrated the %RSD value in the range of 0.1136 – 0.7084%. The results revealed that the proposed method presents good precision with the %RSD value of ≤2%.

LOD is the lowest concentration of analyte in a sample which can be identified but not necessarily quantified. Meanwhile, LOQ is the minimum concentration of analyte which can be calculated with suitable precision. Both the LOD and LOQ can be measured based on standard deviation of y-intercepts and slope value acquired from the calibration curve. The LOD and LOQ of the proposed method were 4.0535 and 13.5118 μg/mL, respectively. The pH, materials, and process of the study in the SIF medium employed in the system was evident not to interfere with the analytical process, as displayed by the acceptable values of accuracy and precision. The analytical method developed in this study also provided adequate sensitivity ad illustrated by the LOD and LOQ values. Aggregation was not presented in the study, which suggested any procedures implemented in the study that were triggering any precipitation and chemical conjugation.

CONCLUSION

A simple, accurate, and precise method for the analysis of quercetin in nanoemulsion preparations by implementing UV/Vis spectrophotometric was developed. The validation result revealed that the procedure was linear, accurate, and precise, with the value of each parameter complied with the limits according to the ICH guidelines. In vitro drug release of quercetin loaded-nanoemulsion was investigated by applying UV/Vis spectrophotometric at a certain time interval, displaying an increased quercetin release profile from 0 to 360 minutes. The UV/Vis spectrophotometric can be utilized to

determine quercetin released from nanoemulsion preparation.

SUGGESTION

In particular for this study, we recommend to develop the experiments in the AGF to conduct the story of the drug release in the GI tract. In our study, we merely incorporated the study in the AIF from the fact that the most massive release and absorption happen in the intestines while in the stomach, it is much less significant. However, it would be astonishing if we could further involve the aspect of stability of the system before acquiring the intestines, with very limited release in the stomach that could be expected, which implies the system to protect quercetin before obtaining its maximum release (and therefore absorption) in the GI tract.

The author would like to thank Thesis Recognition Grant Universitas Gadjah Mada for the funding so that this research could be conducted.

REFERENCES

Abarikwu, S. O., Pant, A. B., & Farombi, E. O. (2012). Dietary Antioxidant, Quercetin, Protects Sertoli-Germ Cell Coculture from Atrazine-Induced Oxidative Damage. *Journal of Biochemical and Molecular Toxicology*, *26*(11), 477–485. https://doi.org/10.1002/jbt.21449

Alva-Ensastegui, J. C, Palomar-Pardavé, M, Romero-Romo, M., Ramírez-Silva, M. T. (2018). Quercetin spectrofluorometric quantification in aqueous media using different surfactants as fluorescence promoters. *RSC Adv*. 8(20), 10980-10986. doi:10.1039/c8ra01213j

Anand David, A. V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy Reviews*, *10*(20), 84–89. https://doi.org/10.4103/0973-7847.194044

Batiha, G. E., Beshbishy, A. M., & Taha, A. (2020). *Polyphenolic Flavonoid : Quercetin*. *March*.

Buchweitz, M., Kroon, P. A., Rich, G. T., & Wilde, P. J. (2016). *Quercetin solubilisation in bile salts: A comparison with sodium dodecyl sulphate. Food Chemistry, 211, 356–364.*doi:10.1016/j.foodchem.2016.05.034

Cai, X., Fang, Z., Dou, J., Yu, A., & Zhai, G. (2013). Bioavailability of Quercetin: Problems and Promises. *Current Medicinal Chemistry*, *20*, 2572–2582.

Changediya, V. V, Jani, R., & Kakde, P. (2019). A Review on Nanoemulsions: A Recent Drug Delivery Tool. *Journal of Drug Delivery and Therapeutics*, *9*(5), 185–191. https://doi.org/10.22270/jddt.v9i5.3577

Firoozeh, N., Mahmoud, O., Layasadat, K., Mohammadreza, A., Esrafil, M., & Ali, K.. (2019). Effects of Quercetin-Loaded Nanoparticles on MCF-7. *Medicina (B Aires)*, 55(114),1-15.

Gao, L., Liu, G., Wang, X., Liu, F., Xu, Y., & Ma, J. (2011). Preparation of a chemically stable quercetin formulation using nanosuspension technology. *International Journal of Pharmaceutics*, *404*(1–2), 231–237. https://doi.org/10.1016/j.ijpharm.2010.11.009

Hua, S. (2014). Comparison of in vitro dialysis release methods of loperamide-encapsulated liposomal gel for topical drug delivery. *International Journal of Nanomedicine*, *9*(1), 735–744. https://doi.org/10.2147/IJN.S55805.

International Conference on Harmonization (ICH), (1995), Q2A: Text on Validation of Analytical Procedures.

International Conference on Harmonization (ICH), (1997), Q2B: Validation of Analytical Procedures: Methodology.

Madiha, S., Batool, Z., Tabassum, S., Liaquat, L., Sadir, S., Shahzad, S., Naqvi, F., Saleem, S., Yousuf, S., Nawaz, A., Ahmad, S., Sajid, I., Afzal, A., & Haider, S. (2021). Quercetin exhibits potent antioxidant activity, restores motor and non-motor deficits induced by rotenone toxicity. *PLoS ONE*, *16*(11 November), 1–26. https://doi.org/10.1371/journal.pone.0258928

Nguyen, L. T., Lee, Y. H., Sharma, A. R., Park, J. B., Jagga, S., Sharma, G., Lee, S. S., & Nam, J. S. (2017). Quercetin induces apoptosis and cell cycle arrest in triple-negative breast cancer cells through modulation of Foxo3a activity. *Korean Journal of Physiology and Pharmacology*, *21*(2), 205–213. https://doi.org/10.4196/kjpp.2017.21.2.205.

Niazvand, F., Orazizadeh, M., Khorsandi, L., Abbaspour, M., Mansouri, E., & Khodadadi, A. (2019). Effects of Quercetin-Loaded Nanoparticles on MCF-7 Human Breast Cancer Cells. *Medicina (Kaunas,*

Lithuania), *55*(4), 114. https://doi.org/10.3390/medicina55040114.

Pangeni, R., Kang, S. W., Oak, M., Park, E. Y., & Park, J. W. (2017). Oral delivery of quercetin in oil-in-water nanoemulsion: In vitro characterization and in vivo anti-obesity efficacy in mice. *Journal of Functional Foods*, *38*, 571–581. https://doi.org/10.1016/j.jff.2017.09.059

Ranganathan, S., Halagowder, D., & Sivasithambaram, N. D. (2015). Quercetin suppresses twist to induce apoptosis in MCF-7 breast cancer cells. *PLoS ONE*, *10*(10), 1–21. https://doi.org/10.1371/journal.pone.0141370

Patel, M. K., Shah, S. K., Tyagi, C. K., & Usman, M. R. M. (2020). Method Development And Validation for Estimation Of Quercetin Using UV and RP-Hplc In Bulk And Formulation. *Plant Archives,* 20(2), 4343-4347.

Patil, V., Angadi, S., & Devdhe, S. (2015). Determination of quercetin by UV spectroscopy as quality control parameter in herbal plant: *Cocculus hirsutus.* Journal of Chemical and Pharmaceutical Research, 7(1), 99-104.

Priprem, A., Watanatorn, J., Sutthiparinyanont, S., Phachonpai, W., & Muchimapura, S. (2008). Anxiety and cognitive effects of quercetin liposomes in rats. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *4*(1), 70–78. https://doi.org/10.1016/j.nano.2007.12.001

Rauf, A., Imran, M., Khan, I.A., ur-Rehman, M.-, Gilani, S. A., Mehmood, Z., & Mubarak, M.S., (2018), Anticancer potential of quercetin: A comprehensive review, *Phytotherapy Research,* **32**(11): 2109-2130.

Son, H.Y., Lee, M.S., Chang, E., et al. (2019). Formulation and characterization of quercetin-loaded oil in water nanoemulsion and evaluation of hypocholesterolemic activity in rats. *Nutrients*. 11(2). doi:10.3390/nu11020244

Spagnuolo, C., Cerella, C., Russo, M., Chateauvieux, S., Diederich, M., & Russo, G. L. (2011). Quercetin downregulates Mcl-1 by acting on mRNA stability and protein degradation. *British Journal of Cancer*, *105*(2), 221–230. https://doi.org/10.1038/bjc.2011.229

Tang, S. M., Deng, X. T., Zhou, J., Li, Q. P., Ge, X. X., & Miao, L. (2020). Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects. *Biomedicine and Pharmacotherapy*, *121*(July 2019), 109604. https://doi.org/10.1016/j.biopha.2019.109604

Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules*, *24*(6). https://doi.org/10.3390/molecules24061123

Yang, D., Wang, T., Long, M., & Li, P. (2020). Quercetin: Its Main Pharmacological Activity and Potential Application in Clinical Medicine. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/10.1155/2020/8825387

Yunita, E., Yulianto, D., Fatimah, S., & Firanita, T. (2020). Validation of UV-Vis Spectrophotometric Method of Quercetin in Ethanol Extract of Tamarind Leaf. *Journal of Fundamental and Applied Pharmaceutical Science*, *1*(1). https://doi.org/10.18196/jfaps.010102