RESEARCH ARTICLE

e-ISSN: 2717-7904
https://doi.org/10.37662/jpt.2022.1000275

Journal of Pharmaceutical Technology

Development and validation of an HPLC method for determination of quercetin

Deniz Onan[1], Tilbe Çevikelli[2], Umay Merve Güven[2], Esra Demirtürk[2*]

[1]Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Adana, Turkey
[2]Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Adana, Turkey

ARTICLE INFO

Article history:

Received 24 Sep 2021
Revised 07 Mar 2022
Accepted 30 Oct 2022
Online 07 Nov 2022
Published 07 Nov 2022

Keywords:

Quercetin
microemulsion based gel
validation
HPLC

**Corresponding author:*
demirturkesra@hotmail.com

ABSTRACT

This study validated a high performance liquid chromatography (HPLC) method for the determination of quercetin (QE) in microemulsion (ME) based gel formulations. The analyses were performed on a C18 column (150x4.6 mm, 5 µm particle size) at room temperature with UV detection at 254 nm. The mobile phase was composed of methanol:water (65:35, v/v, 2% acetic acid) mixture, and flow rate was set to 1 mL/min. The method was validated according to the international guidelines with respect to linearity range, stability, limit of quantitation and detection, precision and accuracy. The method was linear within 5 – 100 µg/mL range with a correlation coefficient of 0.9995. The intra- and inter-assay precisions presented RSD values lower than 2%. The method reported is a fast and reliable HPLC method useful for QE determination in ME based gel formulations.

1. INTRODUCTION

QE is readily available in fruits and vegetables and is preferred because of its unique biological properties that can improve mental/physical performance and reduce the risk of infection [1]. Flavonoids are very promising drug candidates because of their ability to scavenge radicals. Considering its anti-inflammatory, antiviral and antioxidant activities and its ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, its potential benefits for general health and disease resistance are revealed [2].

QE has been formulated and characterized in many dosage forms such as nanoemulsion, liposome, lipid nanoparticle, nanostructured lipid carrier and solid lipid nanoparticle [3-7]. Poor solubility and stability properties of QE occurs difficulties during formulation design. Due to lipophilic cyclic structure of QE, its partition coefficient is 1.82 ± 0.32 [8, 9]. However, despite this log P, penetration into the skin is limited due to quercetin's polar hydroxyl groups. In order to increase the absorption of QE through the skin, various dosage forms have been tried and evaluated with in vitro-in vivo characterizations. Its poor water solubility suggested the presence of a lipid phase to increase the solubility of QE in the formulation. On the other hand, QE polar heads favor water presence, so quercetin can localize at the interface [8]. Microemulsion (ME) is defined as a dispersion consisting of oil, surfactant, cosurfactant and aqueous phase, which promising thermodynamically stable liquid systems with a droplet diameter usually within the range of 10-100

nm. However, the liquid form and low viscosity of MEs limitates adhere to the surface of the skin and maintain long time on the application area [10, 11]. In order to overcome these limitations and prolong the functioning time on the skin, gelling agents were used to obtain proper viscosity for topical application. MEs provide significant advantages on improving drug delivery through transdermal and dermal routes of administration; as the have solubilizing ability for poorly water soluble drugs, convenient particle size and permeation ability across skin membrane [12]. However, since the viscosity plays an important role in promoting drug retention at the application site, microemulsions have a considerable limitation [13]. Microemulsions are low-viscous liquids and are subject to dosing errors during application due to their vicosity, beside allowing limited drug retention at the skin [14]. To overcome these limitations, incorporating microemulsions into gelling systems, enhances the application. In order to increase viscosity, improve application on the skin and to prolong the drug retention at the application site, Carbopol based gels provide great advantages as reported in the literature [15].

Up to now, HPLC and spectrophotometric methods have been the most common techniques which have been utilized for the determination of quercetin [16-19]. In this study, a simple and reliable HPLC method was developed and validated for in vitro determination of QE from the ME based gel formulations.

2. MATERIALS AND METHODS

2.1. Materials

QE was obtained from Sigma Aldrich (USA). Ethanol, disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) were purchased from Merck (Germany). Deionized water was obtained from Mili-Q water system, that is used for preparation of buffers and standard solutions. All other chemicals were reagent grade and used without further purification.

2.2. Methods

2.2.1. Instrumentation

Chromatogrphic system was carried out using isocratic mode on Shimadzu Nexera 2. C18 column 250 x 4,6 mm, particle size 5 µm was used (GL Sciences). The mobile phase consisting 65% methanol: 35% purified water(v/v) mixture with 2% acetic acid. The flow rate was set at 1 mL/min with 20 µL as injection volume. Detection wavelength was 254 nm at 25°C and retention time was 6.5 min [19].

2.2.2. Standard Solutions

The stock solution of quercetin (100 µg/mL) was prepared in ethanol. pH 5.5 phosphate buffered saline:ethanol (60:40) (v/v) mixture was used to obtain standard solutions within the concentration range of 5-100 µg/mL

2.2.3. Method Validation

Method validation studies of Quercetin was carried out occording to the International Council on Harmonization (ICH) Guideline Q2(R1). [20] The described method was validated as linearity, accuracy, precision (intra-day precision, inter day precision), sensitivity and stability.

2.2.4. Linearity

The linearity of the method was determined by selected eleven concentration points which were 5, 10, 15, 20, 30, 40, 80 and 100 µg/mL. The calibration equation evaluated by determination coefficient, slope and intercept.

2.2.5. Precision

The inter- day precision studies were carried out for three different concentrations (5, 40, 80 µg/mL) within calibration range analysed 3 consecutive days. Intra-day precision studies also carried out with same 3 concentrations within the same day. All results evaluated by mean, standard deviation (SD) and relative standard deviation (RSD %) values.

2.2.6. Accuracy

The accuracy of the method was assessed by performing the recovery study. It was conducted by

adding known amount of quercetin. Three different concentrations of quercetin (5, 40, 80 µg/mL) added to placebo formulation as final concentration of active substance will be 0.5%.

2.2.7. Sensitivity

Limit of detection (LOD) is the minimum concentration of analyte which can be detected in a sample. Limit of quantitation (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy under the specified conditions, to evaluate the sensitivity of method.

Besides, LOD (the lowest QE concentration that can be detected but not quantitated in the sample) and LOQ (the lowest QE concentration in the sample that can be measured with suitable precision and accuracy) were calculated using the following equations (σ= the standard deviation (SD) of the intercept; S=the slope of the calibration curve).

$$LOD = 3.3x\left(\frac{\sigma}{S}\right) \quad \text{Equation 1}$$

$$LOQ = 10x\left(\frac{\sigma}{S}\right) \quad \text{Equation 2}$$

2.2.8. Stability

The stability of samples at three different concentrations (5, 40, 80 µg/mL) were evaluated during test conditions. Results evaluated by mean, SD and RSD % compared to initial concentrations.

3. RESULTS AND DISCUSSION

3.1. Optimization of Chromatographic Conditions

It was determined that QE has a maximum absorbance in 254 nm wavelength. Column selectivity for the separation of all related substances is critical. QE was well retained and separated with comparatively sharp peaks using the C18 column (4.6 x 250 mm, 5 µm particle size) (Figure 1). The optimum conditions for the HPLC method were given in Table 1.

3.2. Validation of the Method

3.2.1. Linearity

Linearity of the method for quercetin evaluated by calibration equation and determination coefficient. According to calibration curves the method was found linear wirthin the concentration range of 5-100 µg/mL. Determination coefficient was over 0.999 as demonstration of linearity.

Table 1. *Optimum conditions for HPLC analysis*

Column	C18 (4.6 x 250 mm, 5 µm, GL Sciences)
Mobile phase	Methanol:Water (65:35) (v/v) + 2% acetic acid
Wavelength	254 nm
Temperature	25°C
Injection volume	20 µL
Flow rate	1 mL/min
Retention time	6.5 min

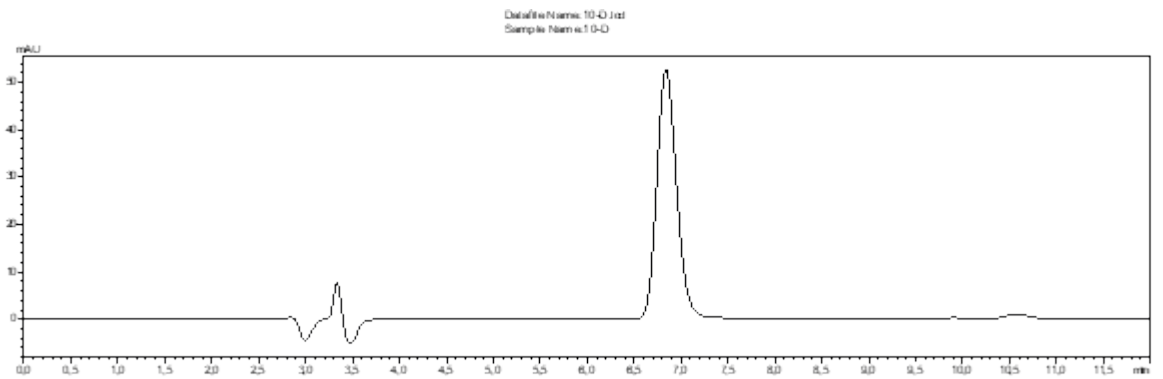

Figure 1. *HPLC chromatogram of QE (10 µg/mL)*

Table 2. *The linearity data of the developed method (n=6)*

Calibration equation	y = 78242x + 49282
Determination coefficient (r2)	0.9995
Linearity range (µg/mL)	5-100
Number of points	8
LOQ (µg/mL)	10.65
LOD (µg/mL)	3.52

3.2.2. Sensitivity

The sensitivity of the analytical method was evaluated by determining LOD; (signal to noise ratios of 3:1) and LOQ; (signal to noise ratios of 10:1). The LOD value of 3.52 µg/mL and LOQ value of 10.65 µg/mL were verified the sensitivity of the analytical method (Table 2). LOQ was taken as lowest concentration of QE that could be quantitively determined with acceptable accuracy and precision

3.2.3. Accuracy

The accuracy of the method was evaluated at 3 different concentrations (5, 40, 80 µg/mL) as a result of recovery calculation. The RSD % values which is less than 2% was taken as an indication of sufficient accuracy of the developed method.

3.2.4. Precision

Inter-day and intra-day precision studies evaluated by mean, SD and RSD % values. The RSD % values which is less than 2% was taken as an indication of sufficient precision of the developed method.

3.2.5. Stability

Stability of standard solutions during analyses were evaluated at 3 concentrations (5, 40, 80 µg/mL). Results evaluated by evaluation of mean, SD and RSD % compared to initial concentrations.

Table 3. *Accuracy results*

Added Concentration (5, 40, 80 µg/mL)	**Measured Concentration (µg/mL)**			**Percentage (%)**		
	5.15	40.03	80.12	103.00	100.08	100.15
	5.09	40.10	80.09	101.80	100.25	100.11
	5.11	40.03	80.11	102.20	100.08	100.14
	5.10	40.07	79.91	102.00	100.18	99.89
	4.96	40.09	80.01	99.20	100.23	100.01
	4.98	40.11	80.12	99.60	100.28	100.15
Mean	5.07	40.07	80.06	101.30	100.18	100.08
SD	0.08	0.03	0.08	1.53	0.09	0.11
RSD(%)	1.51	0.09	0.11	1.51	0.09	0.11

Table 4. *Intra day precision results*

Concentration (µg/mL)	**5**	**40**	**80**
	5.11	40.03	80.03
	5.12	40.07	80.08
	5.09	40.12	80.10
	4.92	40.12	80.01
	5.05	40.03	79.98
	5.11	40.14	80.08
Mean	5.07	40.09	80.05
SD	0.08	0.05	0.05
RSD (%)	1.50	0.12	0.06

Table 5. *Inter day precision results*

Concentration (µg/mL)	**5**	**40**	**80**
	5.11	40.12	80.15
	5.13	40.13	80.15
	5.15	40.14	79.89
	5.09	39.91	80.05
	5.12	39.97	80.10
	5.16	40.14	80.15
Mean	5.13	40.07	80.08
SD	0.03	0.10	0.10
RSD (%)	0.50	0.25	0.13

Table 6. *Stability Results*

Time (h)	Concentration (µg/mL)		
	5	40	80
0	5.04±0.03 (0.60)*	40.04±0.02 (0.05)*	80.03±003
24	5.01±0.04	40.03±0.03	80.01±0.04
48	4.98±0.06	40.00±0.05	79.98±0.08

*RSD % values

3.2.6. Application of the method

Pseudo-ternary phase diagrams of microemulsion area were drawn with oleic acid, Kolliphor EL, Transcutol P and water mixtures with surfactant/cosurfactant ratio of 2:1, 1:1, 1:2 [21, 22]. In order to prepare microemulsion based gels, Carbopol 980 1 % was dispersed in purified water with constant stirring to obtain gel base, then the pH was adjusted to 6-6.5 using triethanolamine. Microemulsions were mixed with the gel in 1:1 (w/w) ratio to obtain homogeneous formulation [23].

The particle size and polidispersity index (PDI) of 1:2 ME, 1:1 ME and 2:1 ME were 183,7 nm (PDI:0,292), 179,8 nm (PDI: 0,518) and 441,1 nm (PDI:0,445) respectively [24]. According to in vitro characterization studies, surfactant:cosurfactant ratio 1:1 microemulsion and microemulsion based gel (1:1)(w/w) formulations compared to the pure quercetin during in vitro release studies. Cumulative percentage of quercetin released from formulations determined by the validated HPLC method at predetermined time intervals and carried out for 24 hours. All that results showed that the validated HPLC method can be easily used for the in vitro characterization of QE from microemulsion based gel formulations.

4. CONCLUSION

A new HPLC method was developed and validated for determination and quantification of QE in microemulsion based gel formulations. The method was successfully validated, and all the results obtained confirmed linearity, sensitivity, precision and accuracy of the proposed method. All validation results revealed that it is a preferred method for obtaining reliable data in further experiments such as formulation development and quality control studies for QE.

ETHICAL APPROVAL

Not applicable, because this article does not contain any studies with human or animal subjects.

AUTHOR CONTRIBUTION

Concept: TÇ, DO, UMG, ED; Design: TÇ, DO, UMG, ED; Supervision: TÇ, DO, UMG, ED; Materials: TÇ, DO, UMG, ED; Data Collection and/or Processing: TÇ, DO, UMG, ED; Analysis and/or Interpretation: TÇ, DO, UMG, ED; Literature Search: TÇ, DO, UMG, ED; Writing: TÇ, DO, UMG, ED; Critical Reviews: TÇ, DO, UMG, ED.

SOURCE OF FUNDING

This research received no grant from any funding agency/sector.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

1. Davis JM, Murphy EA, Carmichael MD. Effects of the dietary flavonoid quercetin upon performance and health. Curr. Sports Med. Rep. (2009); 8, 206-213. https://doi.org/10.1249/JSR.0b013e3181ae8959
2. Aguirre LAN, Macarulla MT, Gracia A, Portillo MP. Beneficial effects of quercetin on obesity and diabetes. Open Nutraceuticals Journal. (2011); 4(1), 89-198.
3. Fasolo D, Bassani VL, Teixeira HF, Development of topical nanoemulsions containing quercetin and 3-O-methylquercetin. Die Pharmazie-An International Journal of Pharmaceutical Sciences. (2009); 64(11), 726-730. https://doi.org/10.1691/ph.2009.8357
4. Chessa M, Caddeo C, Valenti D, Manconi M, Sinico C, Fadda AM. Effect of Penetration Enhancer Containing Vesicles on the Percutaneous Delivery of Quercetin through New Born Pig Skin. Pharmaceutics. (2011); 3(3), 497-509. https://doi.org/10.3390/pharmaceutics3030497
5. Scalia S, Franceschinis E, Bertelli D, Iannuccelli V. Comparative evaluation of the effect of permeation enhancers, lipid nanoparticles and colloidal silica on in vivo human skin penetration of quercetin. Skin Pharmacology And Physiology. (2013); 26(2), 57-67. https://doi.org/10.1159/000345210

6. Bose S, Du Y, Takhistov P, Michniak-Kohn B. Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems. International Journal of Pharmaceutics. (2013); 441(1-2), 56-66. https://doi.org/10.1016/j.ijpharm.2012.12.013

7. Bose S, Michniak-Kohn B. Preparation and characterization of lipid based nanosystems for topical delivery of quercetin. European Journal of Pharmaceutical Sciences. (2013); 48(3), 442-452. https://doi.org/10.1016/j.ejps.2012.12.005

8. Rothwell JA, Day AJ, Morgan MR. Experimental determination of octanol-water partition coefficients of quercetin and related flavonoids. Journal Of Agricultural And Food Chemistry. (2005); 53(11), 4355-4360. https://doi.org/10.1021/jf0483669

9. Kajbafvala A, Salabat A, Salimi A. Formulation, characterization, and in vitro/ex vivo evaluation of quercetin-loaded microemulsion for topical application. Pharmaceutical Development And Technology. (2018); 23(8), 741-750. https://doi.org/10.1080/10837450.2016.1263995

10. Yang C, Shen Y, Wang J, Ouahab A, Zhang, Tu J. Cationic polymer-based micro-emulgel with self-preserving ability for transdermal delivery of diclofenac sodium. Drug Delivery. (2015); 22(6), 814-822. https://doi.org/10.3109/10717544.2014.898111

11. Ashara KC, Paun, JS, Soniwala M M, Chavda JR, Mendapara VP, Mori NM. Microemulgel: An overwhelming approach to improve therapeutic action of drug moiety. Saudi Pharmaceutical Journal. (2016); 24(4), 452-457. http://dx.doi.org/10.1016/j.jsps.2014.08.002

12. Shinde UA, Modani S H, Singh KH. Design and development of repaglinide microemulsion gel for transdermal delivery. AAPS PharmSciTech, (2018); 19(1), 315-325.

13. Wan T, Xu T, Pan J, Qin M, Pan W, Zhang G, Xu Y. Microemulsion based gel for topical dermal delivery of pseudolaric acid B: in vitro and in vivo evaluation. International Journal Of Pharmaceutics. (2015); 493(1-2), 111-120. https://doi.org/10.1016/j.ijpharm.2015.07.058

14. Araujo GRS, de Oliveira Porfírio L, Silva LAS, Santana DG, Barbosa PF, Dos Santos CP, Lira AAM. In situ microemulsion-gel obtained from bioadhesive hydroxypropyl methylcellulose films for transdermal administration of zidovudine. Colloids and Surfaces B: Biointerfaces. (2020); 188, 110739. https://doi.org/10.1016/j.colsurfb.2019.110739

15. Pandey SS, Maulvi FA, Patel PS, Shukla MR, Shah KM, Gupta AR, Shah DO. (2020). Cyclosporine laden tailored microemulsion-gel depot for effective treatment of psoriasis: in vitro and in vivo studies. Colloids and Surfaces B: Biointerfaces. (2020); 186, 110681. https://doi.org/10.1016/j.colsurfb.2019.110681

16. Kuntić V, Pejić N, Mićić S, Vukojević V, Vujić Z, Malešev D. Determination of quercetin in pharmaceutical formulations via its reaction with potassium-titanyloxalate: Determination of the stability constants of the quercetin-titanyloxalato complex. Journal of the Serbian Chemical Society. (2005); 70(5), 753-763.

17. Mythili T, Ravindhran R. Determination of Quercetin by HPTLC Method in SesbaniaSesban (L.) Merr. Stem Extract. International Journal of Advences in Pharmacy, Biology and Chemistry. (2013); 2(1), 113-119.

18. Pejic N, Kuntic V, Vujic Z, Micic S. Direct spectrophotometric determination of quercetin in the presence of ascorbic acid. Il Farmaco. (20024); 59(1), 21-24. https://doi.org/10.1016/j.farmac.2003.07.013

19. Casagrande R, Baracat MM, Georgetti SR, Verri Jr WA, Vicentini FT, Rafael JA, Fonseca M J. (2009). Method validation and stability study of quercetin in topical emulsions. Química Nova. (2009); 32 (7), 1939-1942. https://doi.org/10.1590/S0100-40422009000700041

20. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2 (R1). In International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. (2005, October) Switzerland: Geneva.

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

22. Hatahet T, Morille M, Hommoss A, Dorandeu C, Muller RH, Begu S. Dermal quercetin smartCrystals(R): formulation development, antioxidant activity and cellular safety. European Journal Of Pharmaceutics And Biopharmaceutics. (2016); 102, 51-63. https://doi.org/10.1016/j.ejpb.2016.03.004

23. Nikumbh KV, Sevankar SG, Patil MP. Formulation development, in vitro and in vivo evaluation of microemulsion-based gel loaded with ketoprofen. Drug Delivery. (2015); 22(4), 509-515. https://doi.org/10.3109/10717544.2013.859186

24. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. European Journal of Pharmaceutical Sciences. (2003); 18, 113-120. https://doi.org/10.1016/S0928-0987(02)00251-8