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Development of stability indicating RP-HPLC method for Quercetin estimation in Nanostructure liquid crystalline dispersion

Research Article

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Abstract

An attempt has been made to develop stability representing the reverse-phase High-Performance Liquid Chromatography (RP-HPLC) method for quantitative estimation of quercetin Nanostructure liquid crystalline dispersion. HPLC method was developed by C-18 column with the mobile phase comprising of 0.4% orthophosphoric acid [OPA]: Acetonitrile [ACN] (45:55, v/v), isocratically pumped with a rate of flow 1 mL/min and 255 nm detection wavelength. The method validation of HPLC parameters and degradation tests was analysed by International Conference on Harmonization (ICH) guidelines. The developed HPLC method was found to be specific, linear ($r^2 \geq 0.999$) over the selected range of concentration 2-14µg/mL, precise, with the limit of detection (LOD) and limit of quantification (LOQ) as 0.021 and 0.064µg/mL respectively. The entrapment efficiency of quercetin for prepared Nanostructure Liquid Crystalline Dispersion (NLCD) was observed to be 94.55 %. The developed HPLC method was applied for the quantification of quercetin in NLCD. The quercetin peak was completely resolved without any disturbances from degrading products and additives. The developed HPLC method was found to be specific, sensitive, and adequate for the regular assay of marketed products, and NLCD nano formulation containing quercetin.

Keywords: RP-HPLC, Quercetin, Stress degradation, Nanostructure Liquid Crystalline Dispersion.

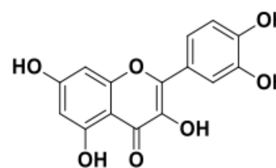
Introduction

In recent times, there has been a notable rise in the use of natural bioactive compounds for addressing chronic illnesses, owing to their minimal toxicity and eco-friendly attributes (1). Quercetin, a naturally occurring flavonoid compound found abundantly in plants, demonstrates diverse pharmacological properties. Due to its broad range of health benefits, quercetin has achieved significant interest from both nutritionists and medicinal chemists (2).

Quercetin a naturally occurring flavonoid have demonstrated different pharmaceutical effects, which includes antidiabetic, anti-inflammatory, wound-healing, hepatoprotective, and antioxidant action (3,4,5). Quercetin, is well known for its antioxidant, anticancer, anti-inflammatory, and antiviral properties. Additionally, it helps with several cardiovascular illnesses (6,7).

Quercetin, often known as 3,3',4',5,7-Pentahydroxy flavone, is a polyphenolic compound found in a variety of fruits and vegetables (8,9,10). Quercetin structure is shown in (figure 1). Quercetin is gaining a lot of attention from researchers due to its widespread availability and strong biological activity as a preventative for a variety of conditions, including neoplasm, cardiovascular diseases, anti-inflammatory, bronchial hyper reactivity, and neurodegenerative disorders. The basic mechanism by which it works is direct radical scavenging of oxygen, either in free or excited form (12,13)

Figure 1. Structure of Quercetin



According to a literature review, there are numerous chromatographic techniques for quantitatively estimating quercetin from various plants. Meanwhile, not many quick HPLC techniques are available for quercetin analysis (13,14). The analyses were carried out on a C18 column with UV detection using 254 nm and at room temperature, according to the most recent method for estimating quercetin. The mobile phase contained a 65:35 (v/v) methanol:water combination with acetic acid (2%) and the flow rate was adjusted to 1 mL/min (15,16).

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The proposed research study objective was to develop and validate an HPLC method for analyzing quercetin that demonstrated stability by ICH (International Conference on Harmonization) standards.

Materials and methods

Materials

Quercetin was received as gift sample from Aktin chemicals, China. In this study HPLC-analytical grade Methanol, Acetonitrile Orthophosphoric acid, Potassium dihydrogen phosphate were used. All additional ingredients were of Pharmaceutical grade.

Instrumentation and Chromatographic conditions

A method was developed by HPLC (Shimadzu HPLC prominence system, LC-20AD, Japan) for quercetin (Aktin chemicals, China). Briefly, the chromatography was carried out using a C18 column (250 X 4.6nm, particle size 5µm, Phenomenex, USA) and the mobile phase used for the elution was Acetonitrile (ACN): 0.4% orthophosphoric acid (OPA) in water. The column temperature was 35°C with a flow rate of 1ml/min.

This developed HPLC method was further used to analyse NLCD and other samples.

Preparation of sample

Primary stock solutions of quercetin in mobile phase was prepared at a concentration of 1 mg/ml. The stock solution is diluted with the mobile phase to produce the standard formulation of the Quercetin drug, which has a concentration range of 2-14µg/ml. Before the HPLC investigation, the standard stock solution was stored at 4 °C to prevent from the degradation, as it is stable at that temperature. Remaining working standard solutions was prepared freshly, and stored at 35°C, in amber-colored light-resistant volumetric flasks with tightly fitting lids during the HPLC analysis (17,18).

Method development

The method development for Quercetin was carried out by experimenting with various proportions of the mobile phase, the concentration of the OPA, temperature, and flow rate

Method Validation for quercetin

The developed approach was validated by ICH recommendations for linearity, sensitivity, suitability of the system, limit of detection, the limit of quantification, precision, accuracy, robustness, and ruggedness.

Linearity

By introducing different amounts of quercetin standard solution, the method's linearity was examined. A stock solution (1 mg/ml) in acetonitrile was created. The right number of dilutions was made using the mobile phase to produce concentrations ranging from 2 to 14 µg/ml (19).

Suitability of the system

It ensures the recognized approach's validity and specificity. The purpose of this test is to determine the repeatability and resolution of the devised method for performing chromatographic analysis. It is a critical step in the method development process. Peak area, retention time (RT), theoretical plates, and tailing factor was among the criteria used to calculate the %Relative Standard Deviation (%RSD). The % RSD of the peak area (less than 2), the N > 2000 value of the theoretical plates and the tailing factor (less than 2) were all within acceptable ranges. This technique verifies that the system appropriateness requirements are met and that the HPLC technology provided is applicable. As observed in the quercetin results, they were sharp, precise, and had good resolution. Results of the aforementioned parameters show that the chosen chromatographic technology is appropriate for further validating and analyzing quercetin (20).

Sensitivity

The quantification limit (LOQ) and detection limit (LOD) were derived from the calibration curve and standard deviation of the reading of the lowest concentration range (Sd).

$$LOD = 3.3 \times \frac{Sd}{Slope} \qquad LOQ = 10 \times \frac{Sd}{Slope}$$

Precision

It determines the specificity and accuracy of the recognized approach. The precision method is designed to determine the repeatability and resolution of the developed method. The precision of the two measurements reflects their similarity. In the current study, inter-day and intra-day assays are subjected to a precision test. The intra-day precision was assessed at various time intervals on the same day, but the inter-day precision was measured across three days. RSD % values were calculated for analytes at low, medium, and high concentrations (2, 4, and 6 µg/ml) (21,34).

Preparation of quercetin loaded NLCD nanoparticles

NLCD nanoparticles loaded with quercetin were prepared by a top-down approach followed by high-speed homogenization. In a separate container the ingredients like Glycerol monooleate (1.8 gm) was taken and melted at 45°C and added 30 mg of quercetin to the molten lipid to form uniform mixture. In another beaker water was taken and preheated at 45°C then poloxamer 407 (Sami labs) was dissolved. The above mixture was then incorporated drop by drop to the preheated water containing stabilizer with constant stirring at the same temperature i.e. 45°C. Then homogenization was performed (IKA-T25, Germany) for 15 min at 16,000 rpm, thereafter probe sonicated (Rivotek, Mumbai) for 5 min. The formulated NLCD was kept for 24, and after equilibrium it was used for further study (22,23).

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Characterization of prepared quercetin loaded NLCD

Particle size analysis

Particle size and PDI (polydispersity index) values of quercetin loaded NLCD batch were analyzed by particle size analyzer (Malvern Zetasizer). The readings were measured at a fixed angle by using a g polystyrene cuvette. The analysis was done after diluting the NLCD formulation with the required amount of milli-Q water (24,25).

Entrapment Efficiency (EE)

The entrapment efficiency of quercetin loaded NLCD was calculated using the centrifugation technique (25°C) at 15000 rpm for 15 min The amount of entrapped quercetin analysed by the high-performance liquid chromatography at 255 nm. (26).

$$\%EE = \frac{\text{Total amount of Drug used in NLCD} - \text{un entrapped drug in NLCD}}{\text{Total amount of drug used in NLCD}} \times 100$$

Transmission electron microscopy

Transmission electron microscopy was performed to observe the dispersion of quercetin loaded NLCD. JEM 1400 JEOL instrument (JEOL, Tokyo, Japan) under high resolution was used to analyze Shape and morphology of quercetin loaded NLCD. Samples were analyzed from STIC Cochin. (27).

Degradation study

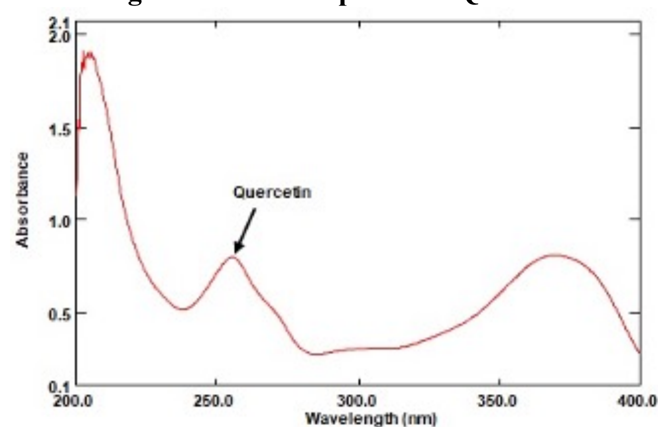
The controlled forced degradation studies of quercetin were conducted under stress conditions that were recommended by the ICH, such as acidic, alkaline, photolytic, oxidative, and thermal environments. Drug solutions were kept in regular storage at time zero and made to deteriorate for two hours. Each of these induced deterioration circumstances led to the development of samples. In an experiment using acid-based degradation, quercetin was subjected to treatment with a 1 mL stock solution of a drug mixture that contained separate solutions of 1 mL of 1 N sodium hydroxide (NaOH) and 1 mL of 1 N hydrochloric acid (HCl) (28,29). Therefore, mentioned solutions were sealed in volumetric flasks, which were then heated for two hours at 80 °C. These two samples were neutralized before analysis. As part of an oxidative degradation experiment, a 30% hydrogen peroxide (H_2O_2) solution was added to 1 ml of the drug mixture stock solution. For the thermal degradation study, 1 mL of the drug combination's stock solutions and 2 mL of the mobile phase were combined in an amber-coloured volumetric flask. The aforementioned solutions were placed in volumetric flasks, sealed, and heated at 80 °C for two hours (30). To analyse chemical degradation, 1 ml of the drug stock solution was added to a clear volumetric flask, the volume was made up to 10 ml using mobile phase. The flask was sealed and left in the light for six hours to test its photochemical stability. The final volume in all of the prior research was made up to 10 ml using mobile phase. After filtering through 0.2 m syringe filters, the sample solution was added to an HPLC chromatographic system for further analysis (30-33).

Results and Discussion

UV-Spectrophotometry

UV spectra were run at 200-400 nm for themethanolic solution of the drug. The spectrum showed maximum absorbance at 255 nm for 10µg/ml solution (Figure 2).

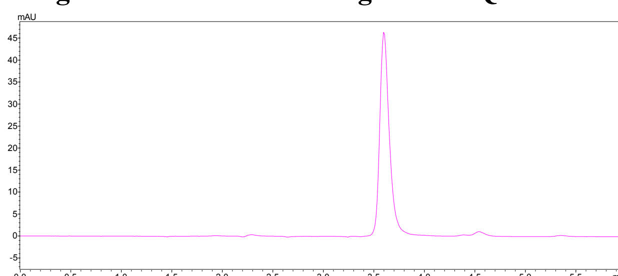
Figure 2. UV-VIS spectra of Quercetin



Method development for quercetin by high-performance liquid chromatography

HPLC analysis was performed for quercetin and the retention time was found to be 3.6 mins at 255 nm (Figure 3).

Figure 3. HPLC chromatogram for Quercetin



Method validation

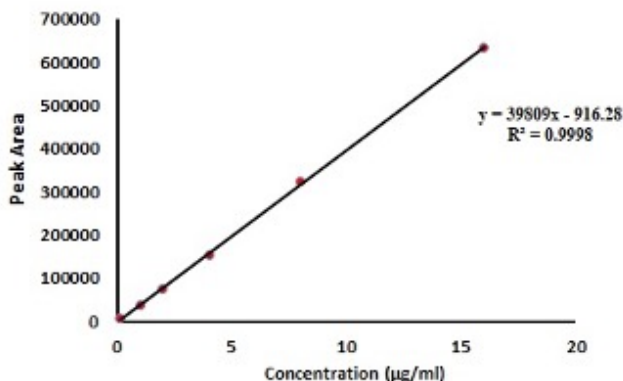
The newly developed method's efficacy and dependability were rigorously validated. According to ICH criteria, under various modified conditions, validation parameters like linearity, system suitability, sensitivity, quantification limit (LOQ), action limit (LOD), precision, accuracy, ruggedness, and robustness are used.

Linearity

The calibration was carried out for three consecutive days of analyzing working solutions at seven distinct levels of concentration. A series of quercetin working sample solutions were prepared (1,2,4,8, and 16 µg/mL) from the mother stock solution. Linearity curve showed regression coefficient (R^2), 0.9998 was shown in figure 4. The reason behind the selection of this concentration is to cover wide concentration rage for the analysis of quercetin form the

developed NLCD, hence the linearity graph shown much gap between the concentration.

Figure 4. Linearity curve for Quercetin



Suitability of the system

The validity and specificity of the suggested technique are ensured by the system's suitability. The test is crucial for method development because it confirms the proposed method's repeatability and resolution for HPLC analysis. The area of the peak, tailing factor, retention time, and theoretical plates were among the parameters whose %RSD was calculated. The parameters like tailing factor (< 2), and theoretical plates (N > 2000) were all within predetermined limits (Table 1). This method thus ensures and fulfill the requirements for system suitability and the correctness of the established method. The peaks obtained for quercetin were found to have good resolution, crispness, and spacing (Figure 3). The results indicate that the chromatographic technique picked is suitable for additional quercetin validation and analysis.

Table 1: Parameters for System Suitability (N=6)

Parameter	Quercetin Mean±SD	Acceptance criteria
Retention Time	3.66 ± 0.005	-
Peak Area	365288 ± 906	-
Plate Count	5392 ± 107	>2000
Tailing Factor	1.355 ± 0.01	≤ 2

Sensitivity

Quercetin's LOQ and LOD were determined to be 0.064µg/ml and 0.021µg/ml, respectively, at their highest concentration (Table 2). The sensitivity of the new technique was determined by comparing its LOQ and LOD values.

Table 2: Data from Linear Regression and Sensitivity Parameters of the developed method

Concentration range (µg/ml)	Slope	Intercept	Regression coefficient (r^2)	LOD (µg/ml)	LOQ (µg/ml)
2-14	39809	916.28	0.9998	0.031	0.091

LOD: Limit of Detection, LOQ: Limit of Quantification

Precision

The interday and intraday precision was assessed by evaluating a selected concentration at three distinct concentrations i.e., 2, 4, and 6 µg/ml preparations of quercetin.

The percentage RSD values for intraday precision varied from 1.60% to 1.86%. For interday precision, the percentage RSD values ranged between 1.12% to 1.93% for quercetin. As a result, %RSD values in both precision studies were less than 2%, meeting the approval standards as shown in Table 3 and demonstrating the new method's high precision.

Table 3: Intraday and Interday Precision of Quercetin

Quercetin Concentratio	Intraday(N =3)	Interday (N=3) RSD (%)		
		First	Second	Third
2	1.60	1.26	1.82	1.12
4	1.28	1.76	1.34	1.93
6	1.86	1.78	1.68	1.53

RSD: Relative Standard Deviation

Accuracy

The accuracy parameter, or percent recovery, was computed by comparing the actual and observed drug concentration values in Table 4. A known concentration of the standard was spiked in triplicate during a recovery test at various concentrations, including 50%, 100%, and 150%.

Table 4: Evaluation of Accuracy Based on Percent Recovery of Quercetin

Active content (µg/ml)	Level of added Quercetin (%)	Recovery (%)	RSD (%)
5	50	98.02	1.92
10	100	101.9	0.96
15	150	101.23	1.69

RSD: Relative Standard Deviation

Indicating that the suggested approach has exceptional accuracy, the mean percentage recovery of quercetin was obtained in the range of 98.02 to 101.9%, respectively. This resulted in a lower% RSD and increased recovery values. The method's applicability for a variety of sample analyses is shown by the high% recovery values attained.

Ruggedness and Robustness

The method was demonstrated to be repeatable, and a low relative standard deviation (R.S.D.) value after recording small intentional changes to the method's operating conditions (flow rate, buffer concentration, mobile phase composition, and change in wavelength) indicated its robustness. The results showed that the retention time remained constant and the %RSD values were (<1). This validates the robustness of the established method, Table 5. This claims to support the durability of the established HPLC method, making it more reliable.

Table 5: Robustness and Ruggedness Evaluation of the Method for Quercetin

Parameter	Changes made	Retention time	%RSD	Plate count ± SD	%RSD
Composition of Mobile Phase [ACN: Phosphate Buffer(0.4% OPA)]	57:43	3.68±0.008	0.21	4030±60	1.49
	55:45	3.66±0.006	0.16	5392±107	1.98
	53:47	3.67±0.007	0.19	5497±99	1.80
Flow Rate	0.8 mL/min	4.56±0.012	0.26	6215±106	1.71
	1 mL/min	3.66±0.006	0.16	5392±107	1.98
	1.2 mL/min	3.04±0.010	0.32	4756±52	1.10
Wavelength	254	3.66±0.006	0.16	5390±99	1.83
	255	3.66±0.006	0.16	5392±107	1.98
	256	3.66±0.010	0.27	5398±80	1.48

SD: Standard Deviation, RSD: Relative Standard Deviation

Preparation and characterization of prepared quercetin-loaded NLCD.

NLCD was prepared in different concentrations of lipid and polymer as shown in table 6.

Table 6: Formulation table for quercetin-loaded NLCD and Blank NLCD

NLCD formulation	Quercetin	Glycerol monooleate (lipid)	Poloxamer 407 (polymer)	Water
Q NLCD 1	30 mg	1.8 gm	600 mg	Upto 30 ml
Blank -NLCD	-	1.8 gm	600 mg	Upto 30 ml

Physicochemical characterization of NLCD

Particle size analysis

The average particle size and zeta potential value for quercetin loaded NLCD batch were124nm and -44.96 mV respectively, with a smaller polydispersity index value 0.27. The particle size for blank NLCD formulation was found to be 113 nm (Table 7). This indicated narrow homogeneous particle size distribution with negative charge on the particle due to free fatty acid content of the lipid.

Table 7: Quercetin loaded NLCD characterization

NLCD formulation	Particle size (nm)	PDI	% EE(%)	Zeta Potential (mV)
Q NLCD	124 ± 5.68	0.271 ± 0.02	94.55 ± 1.26	-44.96 ± 3.74
B NLCD	113 ± 4.93	0.204 ± 0.01	-	-27.0 ± 3.61

n=3; Mean ± Standard Deviation (SD), EE- Entrapment Efficiency, B NLCD - Blank NLCD, Q NLCD - Quercetin loaded NLCD, PDI- Polydispersibility Index

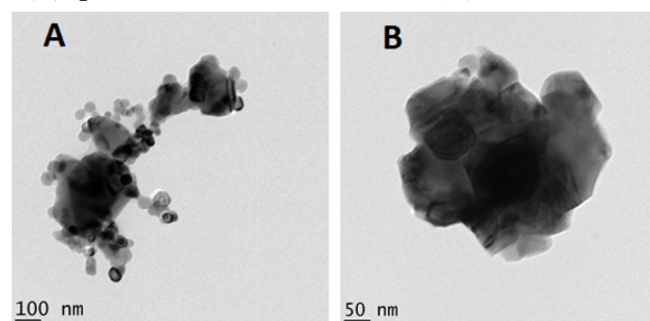
Entrapment efficiency

The entrapment efficiency of the NLCD was calculated using the peak area of the sample analyzedsed by HPLC. NLCD showed good entrapment efficiency of 94.55% for quercetin.

Transmission electron microscopy

NLCDs with irregular hexagonal shapes with less curvature can be seen clearly. Both NLCDs appeared to be cubic, uniform, and have smooth surfaces (Figure 5 A and B).

Figure 5. Transmission electron microscopy images of (A) quercetin-loaded NLCDs and (B) Blank NLCD



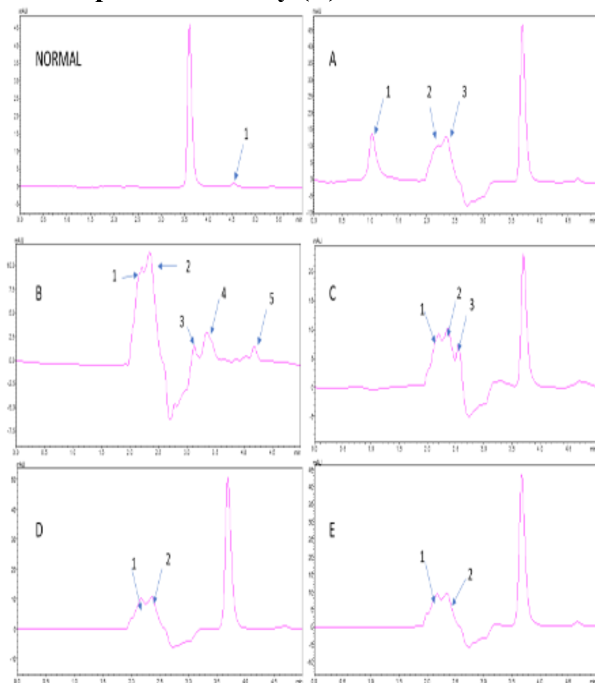
Degradation

Figure 6 displays the outcomes of studies on forced degradation for the analyte matrix. Quercetin analysis revealed small, degraded peaks. The drug was more stable and less prone acidic degradation, with a degradation rate of 12.04%, according to the acid degradation study. In the alkaline degradation study, 86.64% of the quercetin was almost degraded. As a result, it was found that quercetin is the most unstable under alkaline conditions. According to a study, in the thermal degradation of quercetin, there was absence of degradation peaks, indicating a negligible 4.03% degradation. The results of the thermal degradation study showed that heating has no impact on the drug's stability behaviour. Quercetin oxidizes with a 55.84% degradation in the 30% of H_2O_2 degradation study. When the drug was exposed to direct sunlight for 6 hours under photolytic-controlled stress conditions, quercetin was degraded up to 52.82%, which demonstrated light sensitive nature for the quercetin (Table 8). As a result, the drug is vulnerable to degradation once exposed to alkaline conditions, and considerable degrading in oxidative and photolytic conditions as shown by the degradation of the peak. In all degradation conditions, integrity of the peak was maintained with no difference in the drug's retention time.

Table 8: Forced Degradation for Analytes

Degradation of stress study	% Drugs degraded
Acidic (1 M HCl)	12.04
Basic (1M NaOH)	86.64
Thermal	4.03
Oxidative	55.84
Photolytic	52.82

Figure 6. HPLC chromatograms of Quercetin research of forced deterioration under typical, acidic (A), Basic (B), oxidative (C), thermal (D), and photosensitivity (E) conditions.



Comparison with previously published HPLC procedures

According to earlier published procedures, a comparative evaluation of mobile phase (ratios), wavelength, flow rate, and column was performed with the currently validated HPLC method. In comparison to the previously published articles, the current approach, which uses the mobile phase composition0.4% orthophosphoric acid [OPA]: Acetonitrile [ACN] (45:55, v/v), wavelength for detection at 255 nm and flow rate of 1 ml/min, was found to be most sensitive, economical, and stable. The comparative data was shown in Table 9.

Conclusion

To selectively quantify quercetin in freshly prepared NLCD, an HPLC method was devised. The suggested approach was validated for various parameters that were within acceptable range following ICH criteria and found to be simple, quick, more sensitive, and effective. The approach that was created was accurate and cost-effective. With low LOD and LOQ values, the devised method showed high accuracy, precision, and linearity. Additionally, it exhibits well-defined peaks and appropriate drug quantification without excipient contamination in NLCD. Quercetin was found to be stable in higher temperatures, alkaline environments, and oxidative environments, according to forced degradation research. Quercetin, however, was discovered to deteriorate under photolytic circumstances. As a result, the established HPLC method is useful for routine quercetin measurement in any formulation.

Table 9: Comparison between Previously Published Methods

Mobile phase and Flow rate (ml/min)	Temp. (°C)	LOD and LOQ	Column	Retention time (min)	Application	Reference
Acetonitrile and 2% v/v acetic acid (40% : 60% v/v) (pH 2.6) the flow rate of 1.3 mL/ minute,	35°	0.0048 and 0.0390 µg/mL	C-18	3.97	HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicines	23
Methanol–dimethyl sulphoxide (4:1 v/v)	-	LOD-80 ng/ml	C-18	-	Determination of quercetin in human plasma by HPLC with spectrophotometric or electrochemical detection	24
Methanol-acetonitrile-water (60:20:20 v/v/v) flow rate 1.1 ml/min	28⁰C	21.1 and 98.6 µg/mL	C-18	2.30	Quantitative analysis of quercetin in natural sources by RP-HPLC	25
Pure methanol Flow rate 1.0ml/min	35⁰C	1.0 and 4 µg/mL	C-18	2.42	Development and validation of a new RP-HPLC method for the determination of quercetin in green tea	26
0.3% trichloroacetic acid in water and acetonitrile (50:50, *v*/v) Flow rate 0.9ml/min	40⁰C	0.05 and 0.1 µg/mL	C-18	4.5	A new validated HPLC method for the determination of quercetin: Application to study pharmacokinetics in rats	27
Methanol: 0.1% orthophosphoric acid (65:35 v/v) Flow rate 1.0mlm/min	-	0.203 and 0.616 µg/ mL	HiQSil C18HS	8.4	RP-HPLC method development and validation of Quercetin isolated from the plant Tridaxprocumbens L	28

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Conflict of interest

The authors declare that there is no conflict of interest.

Reference

1. Aghababaei, F., Hadidi, M. (2023). Recent advances in potential health benefits of quercetin. Pharmaceuticals. 18;16(7):1020.
2. Wang, G., Wang, Y., Yao, L., Gu, W., Zhao, S., Shen, Z., Lin, Z., Liu, W., Yan, T., (2022). Pharmacological activity of quercetin: an updated review. Evidence-Based Complementary and Alternative Medicine. 2022 Oct;2022.
3. Kumari, A., Yadav, S.K., Pakade, Y.B., Singh, B. and Yadav, S.C., (2010). Development of biodegradable nanoparticles for delivery of quercetin. Colloids and Surfaces B: Biointerfaces, 80(2), pp.184-192.
4. Sgarbieri, V.C. and Pacheco, M.T.B., (2017). Envelhecimentoprematuroooupatológico: longevidade. Brazilian Journal of Food Technology, 20.
5. David, A.V.A., Arulmoli, R. and Parasuraman, S., (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy reviews, 10(20), p.84.
6. Karami, Z. and Hamidi, M., (2016). Cubosomes: remarkable drug delivery potential. Drug discovery today, 21(5), pp.789-801.
7. Geoghegan, F., Wong, R.W.K. and Rabie, A.B.M., (2010). Inhibitory effect of quercetin on periodontal pathogens in vitro. Phytotherapy Research, 24(6), pp.817-820.
8. Fasolo, D., Bassani, V.L. and Teixeira, H.F., (2009). Development of topical nanoemulsions containing quercetin and 3-O-methylquercetin. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 64(11), pp.726-730.
9. Chessa, M., Caddeo, C., Valenti, D., Manconi, M., Sinico, C. and Fadda, A.M., (2011). Effect of penetration enhancer containing vesicles on the percutaneous delivery of quercetin through new born pig skin. Pharmaceutics, 3(3), pp.497-509..
10. Bose, S., Du, Y., Takhistov, P. and Michniak-Kohn, B., (2013). Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems. International journal of Pharmaceutics, 441(1-2), pp.56-66.
11. Shad, J.A., Chinn, C.G. and Brann, O.S., (1999). Acute hepatitis after ingestion of herbs. Southern medical journal, 92(11), pp.1095-1097.
12. Mukherjee, P.K., (2002). Problems and prospects for good manufacturing practice for herbal drugs in Indian systems of medicine. Drug information journal: DIJ/Drug Information Association, 36, pp.635-644.
13. Zeng, Z.D., Liang, Y.Z., Chau, F.T., Chen, S., Daniel, M.K.W. and Chan, C.O., (2007). Mass spectral profiling: an effective tool for quality control of herbal medicines. Analytica chimica acta, 604(2), pp.89-98.
14. Rasheed, A., Reddy, S. and Roja, C., (2012). A review on standardisation of herbal formulation. International Journal of Phytotherapy, 2(2), pp.74-88.
15. FDA, U., (2004). US Food and Drug Administration guidance for industry: botanical drug products.
16. Goppel, M. and Franz, G., (2004). Stability control of senna leaves and senna extracts. Planta medica, 70(05), pp.432-436.
17. Kurangi, B.K. and Jalalpure, S.S., (2018). Review of selected herbal phytoconstituents for potential melanoma treatment. Indian Journal of Health Sciences and Biomedical Research kleu, 11(1), pp.3-11.
18. Kurangi, B., Jalalpure, S. and Jagwani, S., (2019). A validated stability-indicating HPLC method for simultaneous estimation of resveratrol and piperine in cubosome and human plasma. Journal of Chromatography B, 1122–1123, pp. 39–48.
19. Kumar, S., Lather, V. and Pandita, D., (2016). Stability indicating simplified HPLC method for simultaneous analysis of resveratrol and quercetin in nanoparticles and human plasma. Food Chemistry, 197, pp.959-964..
20. Kurangi, B. and Jalalpure, S., (2020). A validated stability-indicating RP-HPLC method for piperine estimation in black pepper, marketed formulation and nanoparticles. Indian Journal of Pharmaceutical Education and Research, 54(3s), pp.s677-86.
21. Kedar, T., Jalalpure, S., Kurangi, B. and Kazi, T., (2022). Development and Validation of Stability-Indicating RP-HPLC Method for the Estimation of Fisetin in Novel Cubosomal Nanoformulation: Application to the Marketed Formulation and Selected Plant Extracts. Current Pharmaceutical Analysis, 18(10), pp.983-992.
22. Chimagave, S.S., Jalalpure, S.S., Patil, A.K. and Kurangi, B.K., (2022). Development and Validation of Stability Indicating UV-Spectrophotometric Method for the Estimation of Hesperidin in Bulk Drugs, Plant Extract, Ayurveda Formulation and Nanoformulation. Indian Journal of Pharmaceutical Education and Research, 56(3), pp.865-872.
23. Chimagave, S.S., Jalalpure, S.S., Patil, A.K. and Kurangi, B.K., (2023). Development and validation of stability indicating RP-HPLC method for estimation of hesperidin in nanotransferosome and Madhiphalarasayana—An Ayurvedic marketed product. Journal of Applied Pharmaceutical Science, 13(2), pp.039-048.
24. Ang, L.F., Yam, M.F., Fung, Y.T.T., Kiang, P.K. and Darwin, Y., (2014). HPLC method for simultaneous quantitative detection of quercetin and curcuminoids in traditional chinese medicines. Journal of pharmacopuncture, 17(4), p.36.
25. Jones, D.J.L., Lim, C.K., Ferry, D.R. and Gescher, A., (1998). Determination of quercetin in human plasma by HPLC with spectrophotometric or electrochemical detection. Biomedical Chromatography, 12(4), pp.232-235.
26. Phani, C.R., Vinaykumar, C., Rao, K.U. and Sindhuja, G., (2010). Quantitative analysis of quercetin in natural sources by RP-HPLC. International Journal of Research in Pharmaceutical and Biomedical Sciences, 1(1), pp.19-22.
27. Savic, I.M., Nikolic, V.D., Savic, I.M., Nikolic, L.B. and Stankovic, M.Z., (2013). Development and validation of a

new RP-HPLC method for determination of quercetin in green tea. Journal of analytical chemistry, 68, pp.906-911.

28. Abdelkawy, K.S., Balyshev, M.E. and Elbarbry, F., (2017). A new validated HPLC method for the determination of quercetin: Application to study pharmacokinetics in rats. Biomedical Chromatography, 31(3), p.e3819.
29. Sanghavi, N., Bhosale, S.D. and Malode, Y., (2014). RP-HPLC method development and validation of Quercetin isolated from the plant Tridax procumbens L. Journal of Scientific and Innovative Research, 3(6), pp.594-597.
30. Patel, R.K., Patel, V.R. and Patel, M.G., (2012). Development and validation of a RP-HPLC method for the simultaneous determination of Embelin, Rottlerin and Ellagic acid in Vidangadichurna. Journal of pharmaceutical analysis, 2(5), pp.366-371.
31. Kudatarkar, N., Jalalpure, S., Balekundri, A. and Kurangi, B., (2021). Analytical method development and validation for estimation of chrysin in chrysin loaded phytosomes using high performance thin layer chromatography. Journal of Liquid Chromatography & Related Technologies, 44(15-16), pp.760-765.
32. Chiprikar, P., Mastiholimath, V. & Balekundri, A. HP-TLC Method Development of Cariprazine Hydrochloride for Applicative Quantification of Nanostructured Lipid Carriers. Chemistry Africa (2023).
33. Nandanwadkar SM, Mastiholimath V. A novel USP-HPTLC protocol compliant method for the simultaneous quantification of E-102, E-124, and E-133 azo dyes in consumer goods. JPC–Journal of Planar Chromatography–Modern TLC. 2020 Aug;33(4):405-12.
34. Kumar, HB, C., Kurangi, B., Soudagar, M., Chimagave, S. ., & Patil, S. . (2023). Development and Validation of UV-Spectrophotometric Method for Estimation of Vinpocetine in Marketed Formulation and Nanoformulation. International Journal of Ayurvedic Medicine, *14*(3), 717–723.

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