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New validated Ultra High Performance Liquid Chromatographic method for Estimation of Quercetin

Research Article

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Abstract

Background: A new Ultra performance liquid chromatographic analytical method was developed and validated for estimation of Quercetin in bulk powder. The reverse phase chromatographic elution using UPLC was carried out in gradient mode on C-18 column (Phenomenex Luna 5 µm, 150 mm X 4.6 mm) as stationary phase utilizing a mobile phase composed of Acetonitrile: 0.1 % formic acid buffer (50:50 v/v) with a flow rate of 1 ml/min and injection volume of 10µl. The analysis was performed at temperature of 40^{0}C and detection of eluent was carried out using photo diode array at 371 nm. The newly developed Ultra performance liquid chromatography method was validated in terms of linearity and range, system suitability, specificity, precision, sensitivity, robustness, ruggedness and accuracy as per ICH guidelines. Results: The retention time of quercetin was found to be at 3.9 min with total run time of analysis is 7 minutes. The linearity was observed between the concentration ranges from 0.5 to 16µg/ml with correlation coefficient 0.999. The precision assays values were found to be less than 2% for both the drugs. The Limit of Detection and Limit of Quantification were 0.47µg/ml and 1.44µg/ml for Quercetin. The mean percentage recovery values were found to be within the range of 90-110 %. Conclusion: The proposed method was found to be simple, specific, precise, sensitive, robust, rugged and accurate and can be used for routine quality control analysis of Quercetin.

Keywords: Quercetin, UHPLC, Photo Diode Array, ICH guidelines.

Introduction

Nobel Prize winner Albert Szent Gyorgyi made the initial discovery of bioflavonoids in the year 1930. Fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine all contain flavonoids, a class of organic compounds with varying phenolic structures. Long before flavonoids were identified as the active ingredients, these natural products were well-known for their positive health effects. Flavonoids come in more than 4000 different kinds, many of which give flowers, fruits, and leaves their appealing colours (1). Quercetin a plant pigment is a potent antioxidant flavonoid and more specifically a flavone, a flavonoid confined in many vegetables and fruits. Mostly in onions, grapes, berries, cherries, broccoli and citrus fruits It is a versatile antioxidant known to possess protective abilities especially against tissue .Quercetin is chemically 2-(3,4- Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Figure 1). Various methods have been developed for estimation of this bioflavonoid due to its growing importance as natural antioxidant .Validated Reverse Phase High Performance Chromatography (RP-HPLC) methods are now widely used in the analysis of pharmaceuticals due to its high degree of accuracy and sensitivity(2). Quercetin possesses a variety of biological actions, including anticancer properties and antioxidant protection against the in vitro oxidation of low-density lipoproteins. According to an in vitro Ames test, quercetin is known to cause Salmonella to mutate. Quercetin is known to be genoprotective against mutagenic agents. In spite of the fact that quercetin is genotoxic to Salmonella in vitro, it is commercially available in western nations. Supplemental quercetin is empirically used to treat allergic diseases such asthma, hay fever, eczema, hives, and gout. Additionally, quercetin has been shown to have positive benefits in clinical studies. Among the flavonoids, quercetin has the strongest pharmacological effects and has the potential to be used therapeutically. It is crucial to ensure its security. The safety of quercetin was examined in the current investigation. for clinical application (3, 4). A bio analytical approach for the simultaneous quantification of both medications in the rat's plasma was developed using RP-HPLC. Using a C-18 reverse phase column, the approach was further validated. Fisetin served as the internal benchmark.

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Fisetin, quercetin, and curcumin were shown to have retention times of 4.2, 5.5, and 12.1 min, respectively. The created approach was discovered to be linear in the 2–10 g/mL range, with regression coefficients (r2) for quercetin, and curcumin of 0.9998 and 0.9998, respectively (5). Ultra performance liquid chromatograpy of quercetin in parenteral administration was studied to quantify in plasma and urine (6). Using methanol as the solvent, a UV Spectrophotometric method was developed to improve and validate the evaluation of apigenin in bulk powder. Apigenin displayed the highest absorbance wavelength at 267 nm. According to each ICH recommendation, this technique has been improved and validated in terms of criteria including specificity, discrimination, linearity, correctness, toughness, and solution stability. Similar to how RP-HPLC was used to quantify apigenin in bulk powder and in its nano formulation, this method produced a sharp peak at 4.3 minutes of retention time with a10µl injection and was found to be linear in the range of 0.5-16 µg/ml with regression coefficient. The detector response for apigenin was linear in the sure concentration range of 2 µg/ml -10 µg/ml.

Methods

Drug Sample: Quercetin was purchased from sigma Aldrich

Chemical and Reagents

All of the analytical-grade chemicals and reagents utilised for the analysis came from Merck laboratories. The Basic Science Research Center of Dr. Prabhakar Kore in Belagavi provided the Milli-Q water that was used.

Instruments used

Shimadzu Prominence LC-30AD UHPLC was utilised. Lab Solutions was the programme utilised. Column C18 (Phenomenex Luna 5 µm, 150 mm X 4.6 mm) was the type of column that was employed. A PDA Detector was used for detection. Shimadzu Uni Bloc was the brand of analytical balance that was utilised. A pH metre from Eutech Instruments was employed, and a Branson 1800 sonicator was used for the sonication.

Method Development

The choice of the stationary phase and the mobile phase systems served as the foundation for the development of the UHPLC method. Quercetin's solubility in various solvents has been tested, and experiments employing diverse mobile phase compositions have been conducted.

In a 500 ml beaker, 0.1% formic acid that had been prepared was added to 500 ml of Millipore water. Pipette 0.1 ml of formic acid into a water-filled beaker with the aid of a calibrated digital pipette. 0.2 Nylon syringe filter was used to filter the mobile phase.

The acetonitrile used to make the mobile phase was filtered using a 0.2 Nylon syringe filter, degassed, then degassed with 0.1% formic acid.

Quercetin Standard Preparation

Accurately weigh 10 mg of quercetin, transferred to a volumetric flask, and added 10 ml of methanol to the flask to make up the volume. 1000 µg/ml of stock solution is produced. 1 ml of the aforementioned stock solution was pipetted into a 10 ml volumetric flask, then added methanol to the flask to get the volume up to 10 ml. 100 µg/ml of stock solution is produced.

Working stock solution of Quercetin: Pipette 1 ml of the stock solution into a 10 ml volumetric flask, then add 10 ml of methanol to the flask to generate a working stock solution with a concentration of 10 µg/ml.

Determination of retention time of Quercetin

The UHPLC was supported by a Hypersil C-18 column as the stationary phase and a mobile phase made up of Methanol: 0.1% Formic Acid (50:50 v/v) as the working standard solution. The working standard solution contained 10 µg/ml of quercetin. The flow rate was changed to 1 ml/min, while 40^0C was kept as the temperature. At 371 nm, UV detection was kept an eye on. The sample that was injected had a volume of 10µl. Retention time was calculated in Fig. 2 after the Chromatogram was obtained.

Validation of UHPLC method [10, 11]

Using selectivity, linearity, precision, accuracy, and robustness characteristics, validation was carried out in accordance with ICH Guidelines to evaluate the performance of the developed UHPLC technique.

System Suitability

To assess the system's precision and accuracy, six replicates of a solution containing an analyte of working concentration were injected.

Linearity

The freshly made linear dilutions were injected into UHPLC in triplicates after being made from a stock solution of quercetin 100 µg/ml. The standard calibration curve was plotted from 0.5 to 16 µg/ml values, according to linearity studies. Concentrations versus peak area were plotted using standard calibration curves. All sample solutions were evaluated in triplicate, and a chromatogram was taken.

Precision

Working standard solutions containing quercetin were injected into the UPLC in three replicates on three separate days and on the same day at 3 various times to demonstrate the accuracy of the procedure.

Robustness

It involved infusing the working standard solution containing quercetin into UHPLC while altering the flow rate and temperature conditions.

Ruggedness

Six duplicates of the working standard Quercetin solution were injected by several analysts on various

days in order to demonstrate the repeatability of the devised analytical procedure.

Limit of Detection and Limit of Quantification.

It was calculated from the standard calibration plot and statistical calculations.

Accuracy

The Standard Addition Method was used to test accuracy at three distinct levels. Quercetin 10 mg was weighed, transferred into a 10 ml volumetric flask, and the remaining 10 ml was filled with methanol to create a stock solution of 1000 µg/ml. Pipette 2.5 ml into a 25 ml volumetric flask, then add methanol to make up the remaining 25 ml to obtain a stock solution containing 100 µg/ml. Pipette 1.5 ml, 3 ml, and 4.5 ml of the aforementioned solution into separate 10 ml volumetric flasks to obtain 15 g/ml, 30 g/ml, and 45 g/ml.

Accuracy of Quercetin at three different levels

- Level-I (50%): An injection was made using a 30 µg/ml stock solution of quercetin that had been spiked with 50%, or 15 µg/ml.
- Level-II (100%): A stock solution of quercetin containing 30 µg/ml was spiked with 100%, or 30 µg/ml, and then injected.
- Level-III (150%): A stock solution of quercetin containing 30 µg/ml was spiked with 150%, or 45 µg/ml, and then administered.

Results and Discussion

Method Development

Quercetin in bulk powder was estimated using a UHPLC technique employing the mobile phase composition. C18 (Phenomenex Luna 5 µm, 150 mm X 4.6 mm) column was used to separate the acetonitrile: 0.1% formic acid (50:50 v/v) mixture. The temperature was held at 40^{0}C and the flow rate was changed to 1 ml/min. At 371 nm, UV detection was seen. The amount of sample that was injected was 10µl. Quercetin retention time was discovered to be 3.9 minutes. Table 1 details the chromatographic conditions, and Figure 2 displays the chromatogram. System Suitability parameters were run and the chromatogram results were obtained. Analysis was done on plate count, tailing factor, resolution, and repeatability. Provided system suitability information in Table 2.

Method Validation

The developed UHPLC technique was found to be specific and selective since no components were eluted from the mobile phase during the analyte's retention time. The gathered information was displayed in Table 3. The working level concentration of quercetin was studied for linearity from 0.5 µg/ml to 16 µg/ml. The linearity regression correlation coefficient was found to be 0.999 and within acceptable bounds. The peak area and retention time % Relative standard deviation was found to be within the limit. Table 4 presented linearity data, while Figure 3 displayed a calibration curve. Several days and periods of time were used for precision. Among the parameters and information in Table 4's data, the mean and % Relative standard deviation were discovered. Modifying the procedure settings allowed for the analysis of robustness. Temperature and total flow were changed, and the results were calculated and examined. The data are shown in Tables 5. Three replicates of the sample solution containing the desired level of the analyte were injected to test the robustness and repeatability. Peak area and retention duration were recorded. The average, standard deviation, and percent Relative standard deviation were all within acceptable bounds. The samples were spiked with three levels of the desired concentration: 50%, 100%, and 150%. We looked at the three replicates for each level. Calculations for theoretical plate, % recovery were conducted. It was found that the mean and % Relative standard deviation were within the limits. In Table 6, accuracy information is displayed. Three different samples were injected, and the best recoveries were 100.5, 101.6, and 99.18%, showing a better degree of technique accuracy. In the suggested method, quercetin was estimated using UHPLC; this is a novel approach in comparison to earlier publications. The experiment was completed using UHPLC because it exhibits superior peak resolution and quicker peak elution. ACN: 0.1% Formic acid is the mobile phase that is employed, and it exhibits superior peak elution and peak retention at 3.9 min. The approach displays a tailing factor of less than 2 and a potential plate count of more than 2000. The LOD and LOQ were found to be 0.47 and 1.44 respectively.

Table 1: Chromatographic Conditions used for UHPLC Analysis

Parameter	Value
Mobile phase	ACN:0.1% Formic Acid
Diluent	Methanol
Pressure	98
Column	BDS Hypersil C18 (250 × 4.6 mm, 5 µm)
Temperature	40^{0}C
Total amt	1ml/min
Injection volume	10µl
UV Wavelength	371nm

Table 2: System Suitability

Sr.no	Retention time	Peak area	Theoretical plate
1	3.9	198648	30020
2	3.9	203317	30453
3	3.9	198920	30360
4	3.9	197356	30532
5	3.9	199520	30154
6	3.9	199445	29512

Table 3: Linearity of UPLC Quercetin

Parameters	Quercetin at 371
Linearity range	0.5-16
Linearity equation	Y= 19281x - 2545.1
Correlation coefficient	0.9982
LOD	0.47
LOQ	1.44
Regression mode	Linear

Table: 4 a Precision (Intraday)

Day/ Time	Concen-tration	Peak area SD	Retention time	% RSD of peak area
9:00 am	0.5	101783.1± 197.205	3.9	1.94
	4	70,794± 571.94	3.9	0.81
	16	316,473.83± 1539.14	3.91	0.48
1:00 pm	0.5	10,226.66± 163.88	3.9	1.6
	4	75,365.16± 503.685	3.91	0.66
	16	3,36,588.33± 2,168.93	3.9	0.64
4:00 pm	0.5	10,368± 184.80	3.9	1.78
	4	75753.83± 444.56	3.9	0.58
	16	316,853.83± 1539.14	3.91	0.48

Table: 4 b Precision (Interday)

Day/Time	Concentration	Peak area ± SD	Retention time	% RSD of peak area
Day 1 (10.00am)	0.5	10173.1± 197.205	3.9	1.94
	4	70,344± 571.94	3.9	0.81
	16	316,853.83± 1539.14	3.91	0.48
Day 2 (10.00am)	0.5	10,157.166± 150.96	3.91	1.49
	4	75532.5± 746.93	3.95	0.991
	16	334,927.83± 1204.30	3.96	0.36
Day 3 (10.00am)	0.5	10354.66± 144.46	3.9	1.4
	4	78698.16± 955.57	3.9	1.21
	16	347504.5± 1797.22	3.9	0.51

Table 5: Analysis of robustness and ruggedness using Quercetin solution

Parameters	Variations	Time(min)	% RSD
Mobile phase	50:50 47:53 45:55	3.93 4.18 3.82	0.255 0.242 0.262
Flow rate	0.8ml/min 1.2ml/min	3.4 4.5	0.17 1.24
Detection wavelength	370 372	4.24 4.20	0.236 0.137
Column oven temp	35 45	3.4 4.7	0.295 0.245
Column	Luna 5u(250*4.6) Luna 5u (150*4.6)	3.93 3.42	0.673 0.877

Table 6: Accuracy

Sr.no	Concentration	Level in %	Added qty	Found qty	Recovery	RSD %
1	2	50	1	1.00595923	100.5959	0.047
2	2	100	2	2.03230642	101.6153	0.12
3	2	150	3	2.97541103	99.18037	0.05

Fig no 1: Quercetin

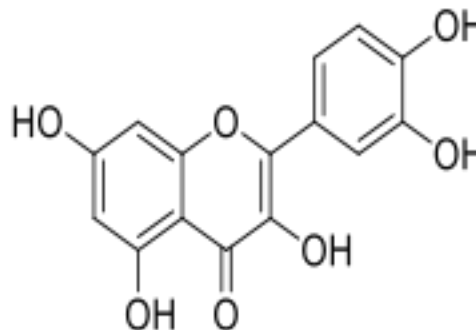


Fig no 2: Chromatogram of Quercetin

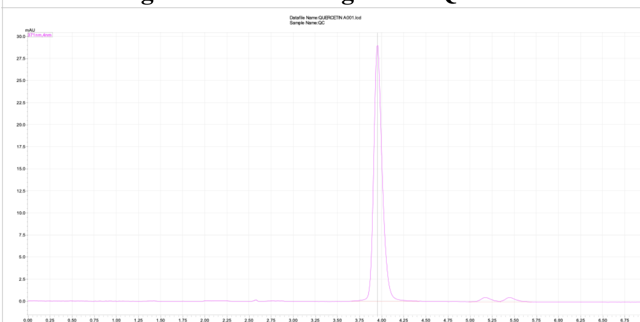
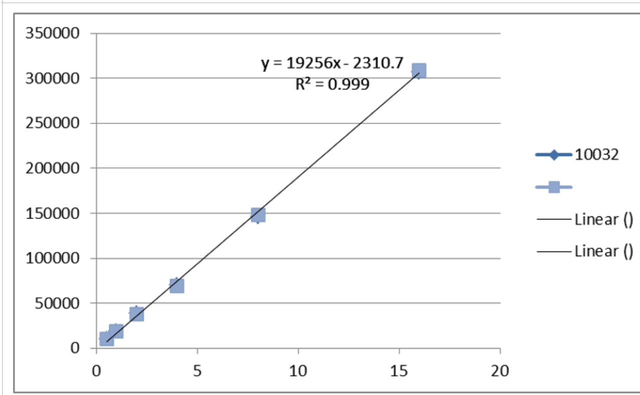


Fig no 3: linearity graph of Quercetin



Conclusion

The UHPLC, which increases the speed, resolution, and sensitivity of the chromatographic analysis while reducing the amount of time, solvent used, and expense, is another of the most important tools in analytical chemistry. There is less noise and a better signal-to-noise ratio in the peaks obtained by UHPLC. For virtually all categories of pharmacological medications, it offers abrupt, narrow peaks. Moreover, it expedites the analysis of complex mixtures, and the peaks generated by this method are more detailed and distinct than those generated by HPLC. A UHPLC method that was developed and validated was used to estimate the amount of quercetin in bulk powder. Although RP C18 had a good Peak Area, Theoretical Plate Count, Retention Time, and Tailing Factor, it was

shown that the analyte was compatible with it when it was utilised. Every parameter was inside the permitted range, according to the validation results of the analysis, which shows that the method was exact, precise, robust, rugged, linear, and accurate.

Abbreviations

UHPLC: Ultra-High-Performance Liquid Chromatographic. ICH: International Council of Harmonisation, UV: Ultraviolet

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