Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Development of a validated RP-HPLC method for estimation of Quercetin

Roma Mathew*[1], Joyamma Varkey[2]

1 College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India.
2 College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India.

ARTICLE HISTORY

Received: 12.01.2021

Accepted: 22.03.2021

Available online: 30.03.2021

DOI:

10.5530/AJPHS.2021.11.1

Keywords:

Quercetin, HPLC, Validation, Method development

*Corresponding author:

Phone : + 91 - 9400852127
Email : romamathew@gmail.com

ABSTRACT

The study aimed to develop a simple, accurate and reproducible method for the estimation of Quercetin using RP-HPLC.A C18 column(250 × 4.6 mm ID, 5µ particle size) with mobile phase consisting of 0.5% O-phosphoric acid: Methanol (50:50 v/v) was used. Quantitative evaluation was performed at 370 nm. The method was validated using ICH guidelines. The developed method is selective, precise and accurate and can be used for routine analysis of preparations in pharmaceutical industry quality control laboratories.

INTRODUCTION

Quercetin, a plant pigment is a potent antioxidant flavonoid and more specifically a flavonol, found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits [1-2] It is a versatile antioxidant known to possess protective abilities especially against tissue injury induced by various drug toxicities[3-5].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 a natural antioxidant [6-8].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 [9,10].

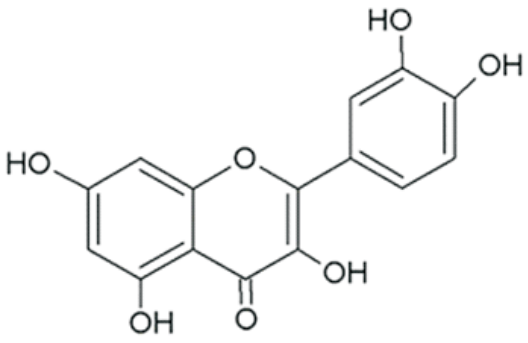

Fig 1 : Structure of Quercetin

MATERIALS AND METHODS

RP-HPLC Method

RP-HPLC instrument equipped with SPD-10AVP UV-vis detector (Shimadzu, Japan), an auto-sampler, Nucleodur, C_{18} (4.6×250mm i.d,5µm particle size) and an LC-solution software.

Standard Stock Solution Preparation

Blank: Diluent was filtered through 0.22µ millipore membrane filters and injected in HPLC system.

Standard stock solution preparation

10 mg drug was accurately weighed and put in to 10ml volumetric flask containing 5 ml of diluents and sonicated for 10 min then the volume was adjusted with diluents up to the mark.

Selection of Mobile Phase and Optimization of Chromatographic condition

Stationary Phase	C_{18}, 250 × 4.6 mm, 5µ particle size, Phenomenex Luna
Elution mode	Isocratic elution mode (50:50 v/v)
Mobile phase	Solvent A was Methanol and Solvent B was 0.5% v/v solution of O-phosphoric acid.
Detector	UV-visible detector
Absorption maxima	370 nm
Column Temperature:	30°7
Flow rate	1.0 ml/min.
Injection volume	20 µl
Diluent	Methanol
Run Time:	15 minutes

Preparation of sample solution

Sample solution of different conc. from 1-10 µg/ml was prepared from above stock solution and diluted with diluents and filtered through 0.22 µ millipore membrane filters and injected in HPLC system.

METHOD VALIDATION

1. Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Selected linearity range for Quercetin was 1-10µg/ml.

A calibration curve was plotted over a concentration range of 1 to 10µg/ml for Quercetin. Accurately measured working stock solution of drug (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1ml) were transferred to separate series of 10ml volumetric flask and diluted up to the mark with methanol. Filtered through 0.2 µm membrane filter and injected for HPLC analysis The Linearity was constructed by plotting concentration against area from each reading.

2. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$LOD = \frac{3.3\sigma}{S}, \qquad LOQ = \frac{10\sigma}{S},$$

Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.

3. Precision

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance or the coefficient of variation.

Repeatability

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of Quercetin (10 µg/ml) was prepared and analysed six times as per the proposed method

Intraday and Interday precision

The intra-day and inter-day variation for determination of Quercetin were carried out Six times in the same day and three consecutive days using concentration 10µg/ml of Quercetin % RSD was calculated.

4. Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 8. The accepted limits of recovery are 90% - 120%.

5.Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and

in parallel, the chromatographic profile was observed and recorded. The studied parameter was Change in wavelength and change in flow rates.

6.Ruggedness

Ruggedness was determined by carrying out analysis of the same samples of Quercetin of concentration 10µg/ml respectively by two different analysts and the respective percentage recovery was noted and the results were indicated as % RSD.

RESULT AND DISCUSSIONS

On HPLC analysis of standard, chromatogram was optimized in which Retention time of drug is shown in Table 1 and Figure 1 (Chromatogram).

Table 1 : Retention time of drug (Quercetin)

S.No.	Name of drug	Retention time (min.)
1.	Quercetin	11.52

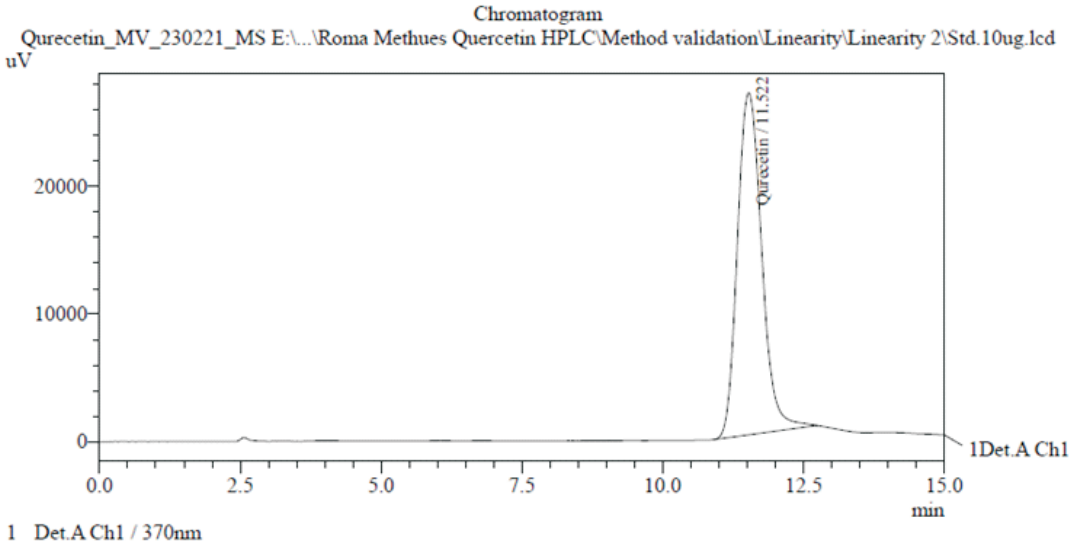

Fig 1 : Chromatogram of Quercetin standard (10µg/ml).

Quercetin (10 µg/ml) chromatogram at Rt = 11.52 minutes

Table 2 : Linearity table of Quercetin

Conc. (µg/ml)	Area 1	Area 2	Area 3	SD	Mean	%RSD	Statistical Analysis
1	98307	97325	98539	98057	644.45636	0.657226	
2	152057	152866	153046	152656.3333	526.783	0.345078	**Intercept** = 896.09
3	220993	228079	228003	225691.6667	4069.3421	1.803054	**Slope** =80824
4	323771	322028	321004	322267.6667	1398.9826	0.434106	**Straight line equation**
5	396584	399426	400136	398715.3333	1879.6173	0.471418	y = 80824x - 896.09
6	484339	483993	491772	486701.3333	4394.7326	0.902963	**Regression coefficient**
7	560588	572620	574053	569087	7395.1419	1.299475	R^2 = 0.998
8	638917	653640	651227	647928	7896.4697	1.218726	
9	734237	732663	737744	734881.3333	2601.0602	0.353943	
10	795892	805749	799435	800358.6667	4992.9933	0.623844	

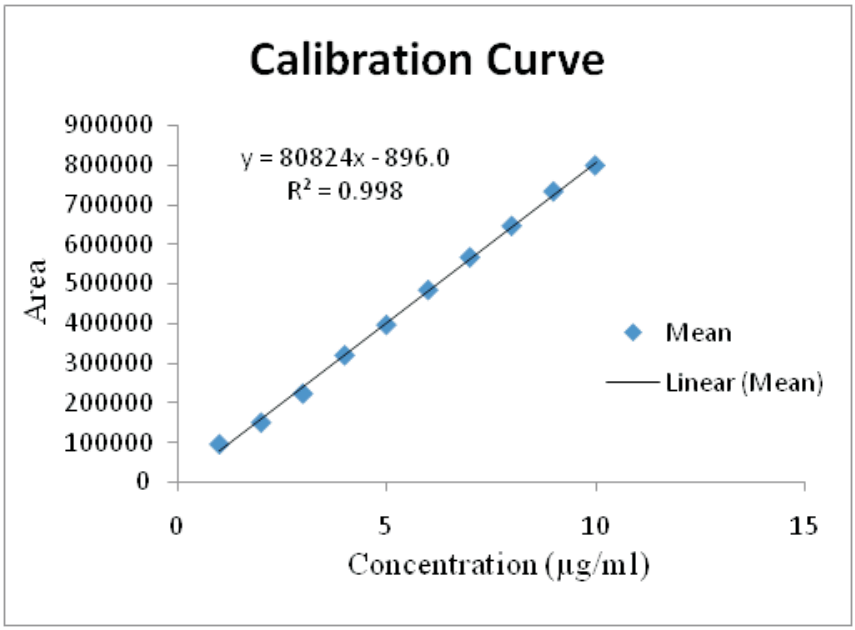

Fig 3 : Standard curve of Quercetin by RP-HPLC

Table 3 : LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Quercetin	0.034	0.104

Linearity regression data, summarized in Table 2, show a good linear relationship between concentration and peak areas over a concentration range of 1-10µg for quercetin (Figure 3).

The Limit of Detection and Limit of Quantification of the method were calculated based on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD was found to be 0.034µg/ml and LOQ was found to be 0.104µg/ml respectively which showed that sensitivity of the method was high. The results obtained were within the limit.

The precision of the method was demonstrated by repeatability, intraday and inter-day variation studies. The mean, standard deviation and the percentage of relative standard deviation were calculated and are presented in above tables. The results reveal that a low relative standard deviation showing that the developed methods are precise.

Observed data (Table 7)of Quercetin are within the required range which indicates good recovery values and hence the accuracy of the method developed.

The robustness study in Table 8,9 indicated that the small change in the conditions did not significantly affect the determination of Quercetin. It was observed that there were no marked changes in the HPLC parameters demonstrating that the HPLC method developed are robust.

Table 4 : Precision Results showing repeatability

Concentration(µg/ml)	% Recovery
10	100.363
10	99.808
10	101.445
10	100.022
10	100.132
10	99.692
Mean	100.244
SD	0.634
%RSD	0.632

Table 5 : Intraday precision

Concentration(µg/ml)	% Recovery
10	99.365
10	98.875
10	98.719
10	100.017
10	99.218
10	99.721
Mean	99.320
SD	0.493864563
%RSD	0.497245505

Table 6 : Interday precision

Concentration(µg/ml)	Area
10	100.668
10	100.306
10	99.376
10	100.443
10	97.649
10	98.824
Mean	99.544
SD	1.165
%RSD	1.170

Table 7 : Accuracy readings of Quercetin

Level of addition	% Recovery	Statistical Analysis		
		Mean	SD	%RSD
	99.42792364	98.7930		
80%	98.90085247			
	98.05039345		0.6950628	0.703554
	98.28949322	98.4438		
100%	98.34417995			
	98.69778779		0.2216354	0.225139
	95.49638721	96.35552421		
120%	96.50372002			
	97.06646541		0.7954608	0.825548

Table 8 : Change in wavelength

368 nm		
Concentration(µg/ml)	Area	Statistical analysis
10	789171	Mean = 787693
10	779828	SD = 7240
10	794080	%RSD = 0.919
372 nm		
Concentration(µg/ml)	Area	Statistical analysis
10	806730	Mean =803813
10	801132	SD = 2806.41
10	803578	%RSD = 0.349

Table 9 : Change in flow rate

0.80 ml/min.		
Concentration(µg/ml)	Area	Statistical analysis
10	1049272	Mean =1048765.667
10	1042868	SD =5661.50
10	1054157	%RSD =0.539
1.2 ml/min.		
Concentration(µg/ml)	Area	Statistical analysis
10	695258	Mean =696915
10	693657	SD =4331.7
10	701831	%RSD =0.621

The ruggedness of the methods was studied by changing the experimental conditions. In the present work, two different analysts performed the method with same set of parameters and mean, standard deviation and relative standard deviation was calculated. It was observed that there were no marked changes in the HPLC parameters demonstrating that the HPLC methods developed are rugged.

Table 10 : Results showing Ruggedness

Analyst 1		
Concentration(µg/ml)	Area	Statistical analysis
10	792056	Mean = 798373.3333
10	803995	SD = 5999.824525
10	799069	%RSD =0.751506128
Analyst 2		
Concentration(µg/ml)	Area	Statistical analysis
10	807434	Mean =814343.6667
10	821796	SD = 7196.362021
10	813801	%RSD = 0.883700864

Table 11 : Summary of the method developed

Parameter	**Result**
Mobile phase	0.5% O-phosphoric acid and Methanol (50:50 v/v)
Injection volume	20 µl
Flow rate	1.0ml/min.
Absorption maxima	370nm
Conc. range	1-10 µg/ml
Correlation coefficient	0.998
Regression equation	y = 80824x - 896.09
Slope	80824
Intercept	896.09
Accuracy (%RSD)	80% (0.703), 100% (0.225), 120% (0.825)
Precision (%RSD)	Repeatability (0.632), Intraday (1.17), Interday (0.497)
LOD µg /ml	0.034576121
LOQ µg /ml	0.104776123
Robustness (%RSD)	Change in absorption maxima, 368nm (0.919), 372 nm(0.349) Change in flow rate, 0.80ml/min (0.539), 1.2ml/min. (0.621),
Ruggedness (%RSD)	Analyst 1(0.751), Analyst 2 (0.883)

The data from the validation of the RP HPLC method developed can be summarised as in table 11.

CONCLUSION

The developed RP-HPLC method was found to be simple, economic, easy, accurate, precise, reproducible and highly sensitive and can be used for routine estimation of Quercetin in bulk.

ACKNOWLEDGEMENT

The authors are grateful to Government Medical College, Thiruvananthapuram for providing necessary research facilities to carry out the research work.

REFERENCES

1. Formica J, Regelson W. Review of the biology of quercetin and related bioflavonoids. *Food and Chemical Toxicology. 1995; 33(12):*1061-1080.
2. Lakhanpal P, Deepak D, Rai K. Quercetin: A Versatile Flavonoid. Internet Journal of Medical Update. 2007; 2(2):20-35.
3. Anand David A, Arulmoli R, Parasuraman S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. Pharmacogn Rev. 2016 ;10(20): 8489.
4. Dengyu Yang, Tiancheng Wang, Miao Long, Peng Li. Quercetin: Its Main Pharmacological Activity and Potential Application in Clinical Medicine. Oxidative Medicine and Cellular Longevity.2020:1-13.
5. Shaik YB,Castellani ML, Perrella A,Conti F, Salini V, Tete S, Madhappan B, Vecchiet J, De Lutiis MA, Caraffa A, Cerulli G. Role of quercetin (a natural herbal compound) in allergy and inflammation. Journal of Biological Regulators and Homeostatic Agents.2006;20(3-4):47-52.
6. Kaur Pawanpreet, Singh Baljeet. Analytical Method Development and Validation of Quercetin: A Review. International Journal of Pharmaceutical and Clinical Research. 2019; 11(2): 49-56.
7. K. Vijaya Sri, J.Vijaya Ratna, A. Annapurna ,B.V.V. Ravi Kumar. Reversed-Phase HPLC Method for Determination of Quercetin in Human Plasma. Asian Journal of Chemistry. 2009;20(1):101-104.
8. Nikita Sanghavi, S. D. Bhosale, Yashwant Malode. RP-HPLC method development and validation of Quercetin isolated from the plant Tridax procumbens L. Journal of Scientific and Innovative Research. 2014; 3(6): 594-597.
9. ICH. Q2A Validation of analytical procedure- Guidelines, Methodology. International Conference on Harmonization. Steering Committee, Geneva. 1994:6-13.
10. The United States Pharmacopoeia (USP 23). National publishing. Asian Ed. Philadelphia. 1995: 291-293

Cite this article :
Roma Mathew, JoyammaVarkey.
Development of a validated RP-HPLC method for estimation of Quercetin.
Asian J. Pharm. Hea. Sci.. 2021;11(1):2415-2422. DOI : 10.5530/AJPHS.2021.11.1