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HPLC Method Development and Validation for Quercetin

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Objective: This research aimed to create an HPLC technique for measuring quercetin, a flavonoid with anti-inflammatory, anti-carcinogenic, and antioxidant characteristics.
Method: Using an Inertsil C18 column of 250 x 4.6 mm and 5 µm, a 70:30 ratio of acetonitrile to water was used as the mobile phase and 1.0 mL/min flow rate. With a retention duration of 2.078 minutes, quercetin first showed at 259 nm. The precision was assessed using a precision assessment of the system and method, and linearity was verified within range of 10 to 50 µg/mL. Three-stage recovery tests were used to establish accuracy, while the calculation of LOD and LOQ was used to establish sensitivity.
Conclusion: The HPLC technique proposed here exhibits great precision with RSD <2%, a very high degree of linearity with R2 = 0.9997, and accuracy across the whole concentration range evaluated. The technique works well for reliably and precisely quantifying quercetin in a range of samples.
Keywords: HPLC, ICH, Quercetin, Method development, Validation.

1. Introduction

The process of separating, identifying, and estimating chemical additives in plant and synthetic materials composed of one or more chemicals or components is known as analytical chemistry. Analytical chemistry can be broadly classified into two categories: quantitative analysis, which measures the concentration of a specific substance in the sample, and qualitative analysis, which identifies the chemicals present in a given sample [i].

Since there are no set procedures, new techniques are developed for testing the innovative product. To increase power and precision, new methods are being developed to reduce the time and expense associated with examining the existence of either non-pharmacopoeial or

pharmacopoeial goods. Their experiments are validated because the methodologies are finalized. Alternatives to the current method, which compares laboratory data based on all the advantages and disadvantages, are created and put into practice [ii]. To determine if the raw materials match the specified criteria and assess the finished product's quality level, the production sector relies on both qualitative and quantitative studies [iii].

Analytical method development is the creation of a specific technique for analyzing drug products from the in-process stage to the finished product, as well as initial validation before routine, investigational, and stability sample analysis [iv]. To find, create, and manufacture pharmaceutical goods, HPLC technique development and validation are essential [v]. The scope of method validation encompasses several validation criteria, such as different approaches that meet different needs. The degree of requirements depending on the intended use determines which analysis method is applied. The known or unidentified issues that occur when employing the validated technique in the regular course are explained. There is less faith in the validated strategy. After developing the method, validation is necessary to ensure that the intended use can achieve the desired level of security [vi].

Over the years, herbal treatments have been a significant source of healthcare for people. According to WHO, eighty per cent of the world's population depends on natural remedies [vii].

Due to the fact that the safety and efficacy of the drugs are directly impacted by the product quality, quality control is a crucial and essential step in the production process of herbal remedies. It is mostly carried out on the finished product as well as on the raw and supplementary materials. The quality of the excipients and raw materials needs to be assessed and tested before the final product is made during the pre-production stage. In order to standardize and ensure the quality of herbal remedies, modern analytical tools are essential. Spectroscopic and chromatographic methods are essential for the analytical validation and quality assurance of herbal treatments [viii].

Flavonoids are naturally occurring secondary metabolites in plants. Quercetin (Figure 1) is a naturally occurring flavonoid derived from many dietary sources such as apples, green tea, tomatoes, red onions, red grapes, black tea, and fresh green leafy vegetables. Quercetin has potential in the treatment of conditions including cardiovascular disease, cancer, and neurological disorders, attributable to its antioxidant and anti-inflammatory characteristics. [ix, x].

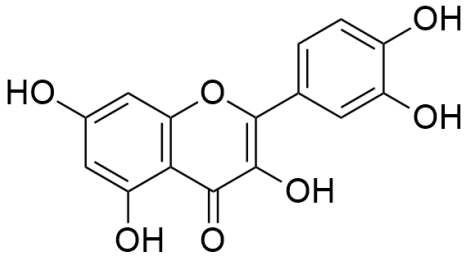

Figure 1: Structure of Quercetin

2. MATERIAL AND METHODS:

Chemicals and Reagents:

Quercetin was obtained from Bio-Molecules India in Khandwa (M.P). The study utilized analytical-grade materials and solvents that conformed to analytical and HPLC standards. The use of high-quality reagents, solvents, and filters is crucial to guarantee the accuracy and reliability of HPLC analysis.

HPLC Method Development

A standard solution was made by precisely measuring 10 mg of quercetin working standard and transferring it to a 100 mL volumetric flask. The stock solution, with a concentration of 1000 µg/ml, was produced by mixing methanol and acetonitrile in a 50:50 ratio. The stock solution underwent further dilution for testing purposes.

Method development wavelength selection:

The UV spectrophotometer was used to scan a blank standard solution including diluents in spectrum mode, covering a wavelength range of 200 to 400 nm. The compound quercetin has a maximum absorption at a wavelength of 259 nm.

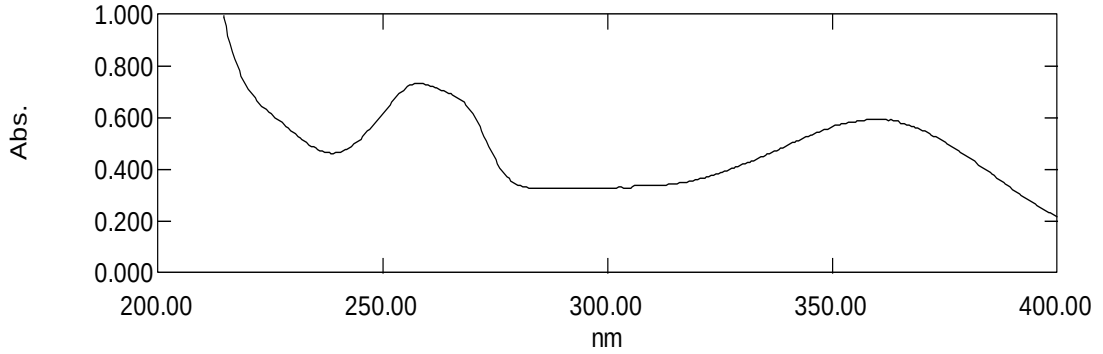

Figure 2: Wavelength Scan

3. Results

Chromatographic conditions:

Table 1 shows the parameters for the chromatographic conditions used in the established technique, while Figure 2 shows the standard chromatogram of the drug.

Table 1: Chromatographic Parameters

Sr. No.	Parameters	Method
1.	Stationary phase (column)	Inertsil C_{18} (250 x 4.6 mm, 5µ)
2.	Detection wavelength (nm)	259 nm
3.	Column temperature (°C)	Ambient
4.	Run time (minutes)	10 min
5.	Flow rate (ml/min)	1.0 ml/min
6.	Mobile Phase	Acetonitrile: Water (70:30)
7.	Volume of injection loop (µl)	20
8.	Drug RT (min)	2.078 min

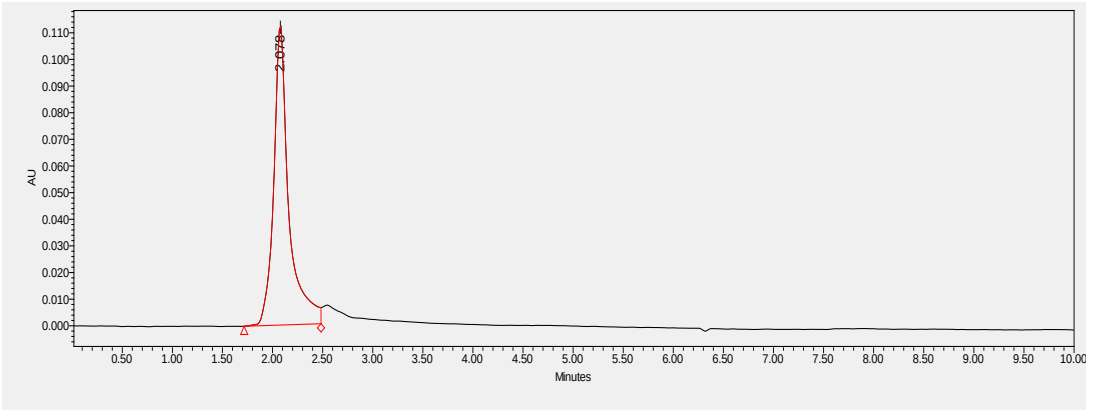

Figure 3: Standard Chromatogram of Quercetin

System Suitability:

It was conducted to confirm that the analytical system is functioning correctly and capable of producing accurate and precise results. Six duplicates of the reference standard with a 10 µg/ml quercetin were individually injected, and a chromatogram was created for each. The outcome are shown in Table 2.

Table 2: System Suitability Parameters

Sr. No.	Parameters	Method
1.	AUC	1177834
2.	No. of Theoretical Plates	3274.000
3.	Tailing Factor	1.246
4.	Retention time	2.078

Linearity:

It was assessed by evaluating five standard solutions (n=5) containing quercetin in the range of 10–50 µg/mL. A concentration versus peak area calibration curve was constructed. The linearity of quercetin was determined by calculating the response ratios of quercetin. The drug response ratio was determined by dividing the absorbance or peak area by the corresponding concentration (Table 3).

Table 3: Linearity parameter

Concentration (µg/mL)	Area
10	1177834
20	2129153
30	3074731
40	4107176
50	5011010
Correlation coefficient (R)	0.999
Regression (r^2)	0.9997
Slope	96443.75
Y-intercept	206668.3

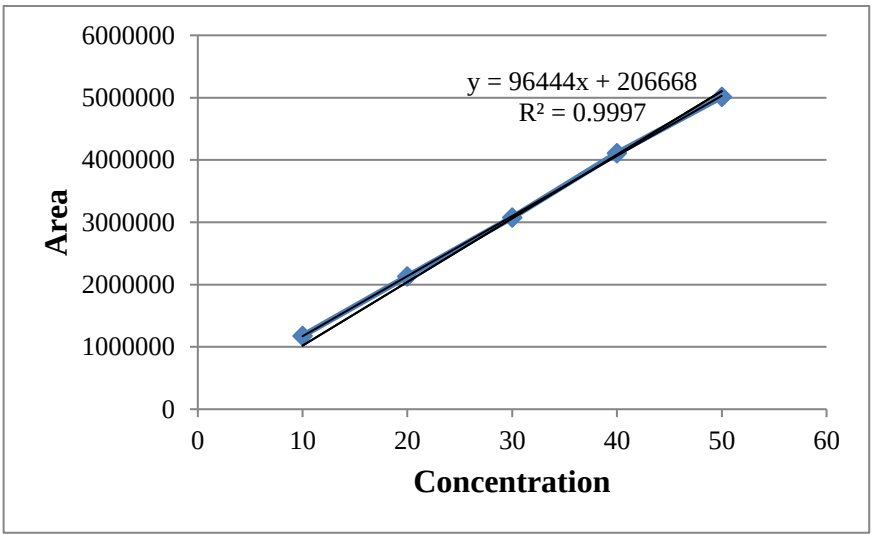

Figure 4: Linearity Response Plot

Accuracy:

The validity and reliability of the proposed techniques were evaluated through recovery experiments conducted at 50%, 100% and 150% as three different levels. The recovery studies were performed by analysis using the methods provided. The mean recovery for quercetin using the reported chromatographic technique was between 98.4% and 99.1%. The amount of substance detected and the percentage of its recovery were calculated. The findings from the utilization studies are shown in Table 4.

Table 4: Results of the recovery study

Level (%)	% Recovery	Mean recovery%
50	98.5	98.4
	98.2	
	98.6	
100	98.9	99.1
	99.4	
	99.1	
150	98.1	98.6
	99.2	
	98.5	
Mean recovery		98.7
%RSD		0.37

Precision:

The accuracy of the drug was assessed based on its repeatability and intermediate precision. The repeatability result shows the level of accuracy achieved when the same operating conditions are maintained within a short period. The mean precision study measures the variability within the laboratory over different days and between different analysts. If the SD %RSD values are both less than 2, this indicates a high level of precision of the method. The result of the accuracy shown is shown in Table 5.

System precision:

Table 5: Result of System precision

Sr. No.	Area
1	5087719
2	5080043
3	5112604
4	5109468
5	5097313
6	5123125
Mean	5101712
% RSD	0.32
Tailing factor	1.1
Theoretical plate	5070

Method precision:

Table 6: Results of method precision

Sample No.	Response	% Assay
1	5086898	99.8
2	5001425	98.1
3	5079720	99.6
4	4994894	98.1
5	5049225	99.0
6	4995213	98.0
Mean		98.9
% RSD		0.82

Limit of Detection (LOD)

It was determined to be 0.120499 µg/ml.

Limit of Quantification (LOQ)

It was found to be 0.32219 µg/mL.

4. Conclusion:

An uncomplicated isocratic HPLC approach has been established for the detection of quercetin. This technique was evaluated for specificity, precision, linearity, accuracy, LOQ and LOD in accordance with ICH criteria. The findings exhibited exceptional sensitivity and selectivity, along with extensive precision, accuracy, and linear range. Furthermore, each parameter demonstrated uniform retention time. This suggested approach is straightforward, efficient, and economical, using ordinary HPLC equipment without necessitating specialised solvents. Consequently, it is appropriate for regular quercetin analysis.

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