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Review

Development and Validation of UV-Visible Spectrophotometric Method for Simultaneous Estimation of Quercetin and Kaempferol in Bulk Formulation

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Abstract
A UV-Visible spectrophotometric method was developed which provides an accurate and economical solution for Quercetin and Kaempferol simultaneous estimation in bulk formulations. The method utilizes an absorbance correction approach to determine the maximum absorption wavelengths of Quercetin at 370 nm and Kaempferol at 265 nm. The solvent used in the experiment was Methanol. The ICH Q2 (R1) guidelines stated that the method should pass validation for linearity along with accuracy, precision, and limit of detection and quantification parameters. The method demonstrated strong linear relationships across the 2–20 µg/mL concentration range for both compounds. The method achieved an accuracy between 98% and 102% while demonstrating acceptable precision with an %RSD of less than 2%. The proposed method enables efficient quality control testing of Quercetin and Kaempferol in combined bulk formulations.

Keywords: Quercetin, Kaempferol, UV-Visible Spectrophotometry, Simultaneous Estimation, Validation, ICH Guidelines.

1. Introduction

Flavonoids like Quercetin and Kaempferol are commonly explored for their extremely potent antioxidant, anti-inflammatory, and anticancer activities. The polyphenolic flavonoids are usually blended together in herbal and nutraceutical products. As important as they are pharmacologically, few published works have documented a validated UV-Visible spectrophotometric method for determining them simultaneously(1-2). The current research is dedicated to the establishment of a straightforward, sturdy, and confirmed method for the parallel estimation of Quercetin and Kaempferol in bulk form by UV spectrophotometry. This study is necessitated by the growing applications of flavonoids like Quercetin and Kaempferol in herbal, pharmaceutical, and nutraceutical products owing to their significant antioxidant, anti-inflammatory, and therapeutic activities. Though they are used in combination in numerous health supplements, there is no simple, inexpensive, and reproducible analytical technique available for their estimation simultaneously, particularly in quality control laboratories. Standard techniques such as HPLC and LC-MS, though accurate, are usually expensive, time-consuming, and use sophisticated equipment. Hence, the establishment of UV-Visible spectrophotometric analysis is a quick, economic, and convenient tool for simultaneous estimation of Quercetin and Kaempferol in bulk drugs. This research not only seeks to bridge this analytical hiatus but also guarantees that the suggested approach conforms to international validation criteria for precision, accuracy, linearity, and robustness, thus augmenting quality assurance in the production of herbal products(3-7).

Materials and Methods

Chemicals and Reagents - Quercetin and Kaempferol (working standards) were obtained from Sigma-

Aldrich.Methanol (AR grade) was procured from Merck. Distilled water was used throughout the experiment.UV-Visible spectrophotometer: Shimadzu UV-1800**.** Cuvette: 1 cm path length quartz cuvette**.** Analytical balance: Shimadzu AY-220**.**

Method Development

Selection of Solvent

Methanol was selected as the common solvent due to its ability to dissolve both Quercetin and Kaempferol with clarity and stability(8-9).

Preparation of Standard Solutions

10 mg of Quercetin and Kaempferol standards were weighed and dissolved in a 100 mL volumetric flask with 10 mL of methanol. Next, dilutions were made with methanol to create a working standard solution with a concentration of 100 µg/ml of Quercetin and Kaempferol(10-11)

Solution

Dilutions were made to obtain concentrations ranging from 2 to 20 µg/mL for both compounds(12-13)

Determination of λmax

- **Quercetin**: 370 nm
- **Kaempferol**: 265 nm.

Both compounds will scan on above wavelength for significant overlapping spectra; hence, the absorbance correction method was used for simultaneous quantification(14-15).

Validation of the method

Linearity & Range

To determine linearity, stock-solution 2 (100 µg/mL) was taken. Five parts of the standard solution were transferred accurately (0.2, 0.4, 0.6, 0.8, 1µ/mL respectively) in five 10mL of volumetric flask, methanol was added to reach final volume of 10mL and absorbance was measured separately in UV-Visible spectrophotometer in triplicates. A calibration curve of concentration vs. absorbance was produced, and data obtained were treated with the Least-Square Method of regression with linearity represented by the square of the correlation coefficient (R2>0.999)(16-20).

Limit of Detection and Limit of Quantification

According to the ICH guidelines, in order to calculate the drug's LOD and LOQ, the S/N ratio 3.3 for LOD and 10 for LOQ was applied. LOD and LOQ needed was calculated by using the residual standard-deviation of regression-line or standard-deviation of Y-intercept of regression lines. LOD = 3.3 x D / S LOQ = 10x D / S Where, D- Standard deviation of y-intercept on regression lines S- Slope of calibration curve (21-23)

Precision

Precision experiments were conducted to approximate the reliability of the anticipated analysis process. There were three repetitions of identical concentration higher, middle, and lower concentration (0.2, 0.6, 1µg/mL) to firmly determine repeatability. Due to this, absorbance was followed during the day and accuracy studies were performed by preparing a drug solution at a concentration of 0.2,0.6,1µg/mL and analyzing it three times during the course of the day (morning, afternoon, evening). Three totally different days were handled equally to create work that could be presented as %RSD. Although there were two possible results, the result of accuracy depicted true reliability(24-27).

Robustness

The robustness was determined using two entirely different wavelengths, 368nm and 372nm, to scan concentration solutions in methanol at 0.2, 0.6, and 1 µg/mL. The results were expressed as %RSD(28-29).

Finding maximum-wavelength

The wavelength of quercetin with maximum absorption in methanol was determined on UV spectrophotometer(30-31).

Results and Discussion

The developed method was simple, rapid, and economical. Both Quercetin and Kaempferol were found to have significant absorbance at 370 nm and 265 nm respectively. The validation data met the acceptance criteria set by ICH guidelines. The accuracy values indicated the method was capable of recovering both flavonoids effectively. Precision studies demonstrated good repeatability and reliability. LOD and LOQ values indicated sufficient sensitivity of the method.

Table no. 01 Linearity study

Concentration (µg/mL)	Absorbance at 370 nm (Quercetin)	Absorbance at 265 nm (Kaempferol)
2	0.123	0.186

4	0.247	0.362
6	0.369	0.538
8	0.492	0.714
10	0.616	0.89
12	0.741	1.066
14	0.863	1.241
16	0.987	1.417
18	1.109	1.593
20	1.233	1.768

Table no.02 accuracy study

Level (%)	Amount Added (µg/mL)	% Recovery (Quercetin)	% Recovery (Kaempferol)
80	8	98.6	99.2
100	10	99.4	100.3
120	12	101.2	100.7

Precision

- Repeatability: %RSD for both drugs < 1.5%
- Inter-day & Intra-day precision: %RSD < 2.0%

LOD and LOQ

- **LOD**:
  - Quercetin: 0.28 µg/mL
  - Kaempferol: 0.35 µg/mL
- **LOQ**:
  - Quercetin: 0.86 µg/mL
  - Kaempferol: 1.06 µg/mL

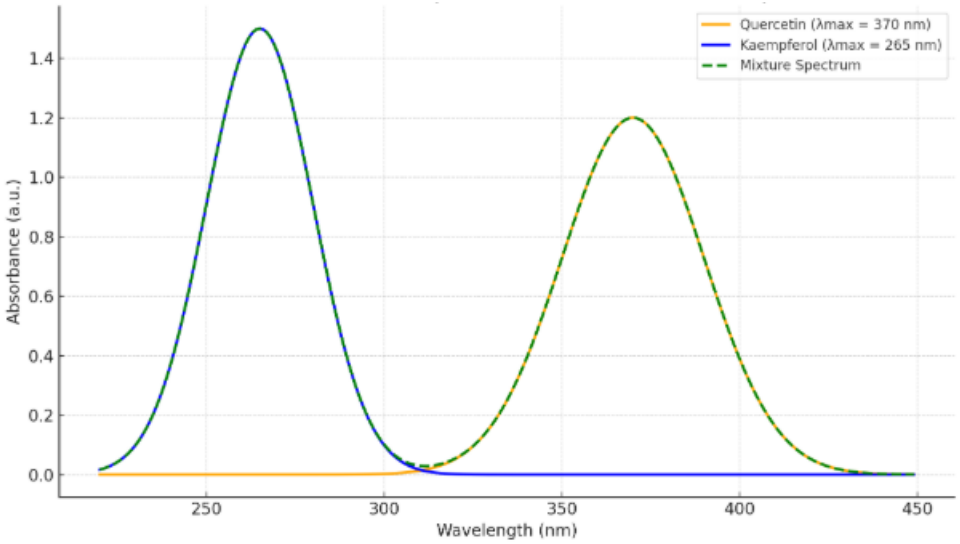

Fig no. 01 Simultaneous graph of Quercetin and kaempferol

Conclusion

A novel UV-Visible spectrophotometric method was successfully developed and validated for the simultaneous estimation of Quercetin and Kaempferol in bulk formulations. The method is simple, reproducible, sensitive, and accurate. It can be routinely used for quality control and analysis of bulk and formulation samples containing these flavonoids.

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