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Development of UV Spectrophotometric Method for Quantitative Estimation of Quercetin in Ocular Formulations

Rabiah Bashir, Tabasum Ali , Shabnam Kawoosa, Nisar Ahmad Khan*
PhD Research Scholar Department of Pharmaceutical Sciences University of Kashmir, J & K India, 190006

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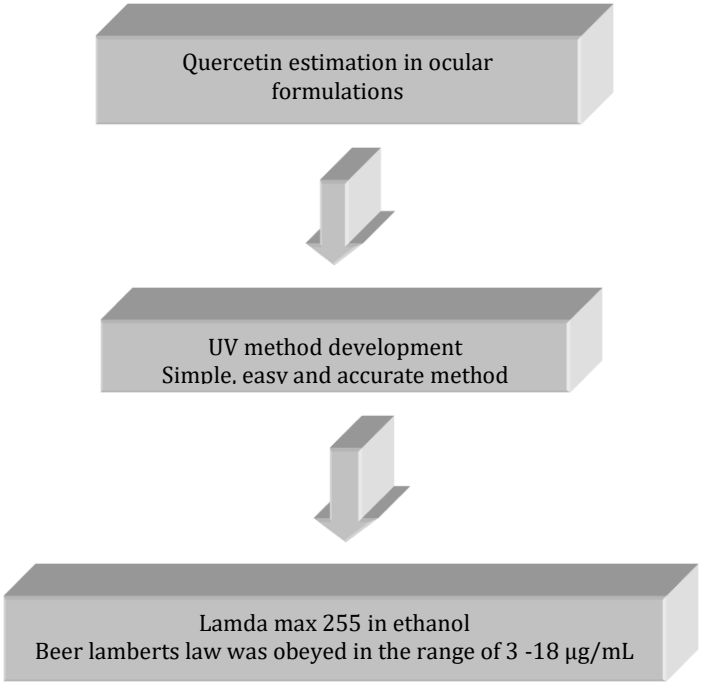
ABSTRACT

To estimate quercetin in ophthalmic formulations, an accurate and simple UV spectroscopic method has been developed. Quercetin shows absorbance maximum at 255 in the ethanol. In the concentration range of 3 -18 µg/mL, Beer Lambert[s] law was obeyed. This method is useful for a regular estimation of quercetin in ophthalmic formulations like eye drops and other formulations are used for the treatment of ophthalmic diseases having quercetin as an active pharmaceutical ingredient which was developed and validated as the underlying method per the guidelines by ICH.

GRAPHICAL ABSTRACT



* Corresponding author: Nisar Ahmad Khan
✉ E-mail: nakhan2008@gmail.com

Introduction

Ocular disease is hazardous to healthiness and lower excellence of existence has overtaken cancer and cardiovascular disease as the third most frequent disease. 34.3 million of citizens were blind worldwide in 2015, another 24.3 million are severely visually impaired and moderate visual impairment in 214 million [1]. People with diseases like corneal disease, cataracts, and eye damage are legally blind, but vision can be regained in such conditions with a proper treatment and prevention. However, it takes a lot of money and time putting a great financial and material strain on patients. Recently, ocular surgery, laser, and medications remain the three primary techniques for treatment of ocular problems, but treatment with drugs continues to have an essential responsibility in diagnosing, treating, and preventing ocular diseases [2, 4]. Quercetin also known as 3, 3', 4', 5, 7 penta hydroxy flavanol is commonly present in a wide range of vegetables and fruits which is recently of high significance. Quercetin and its glycosides has been the subject of numerous investigations. Quercetin has strong anti-oxidant, anti-inflammatory, anti-fibrotic, immunomodulatory, anticancer, and other biological activities as well as other uses for cure of both bacterial and viral infections, tumors, high blood sugar levels, high lipid states, and diseases of immune system [5, 10]. Anti-inflammatory, anti-oxidant, and other actions of quercetin; in particular, have an effect on treating keratoconus, conjunctivitis, cataracts, dry eye disease, and other ocular disorders [11, 15].

Because of the vast spectrum of therapeutic effects of quercetin in the treatment of ocular illnesses, a method for estimating quercetin in ocular formulations should be developed and validated. The goal of this study is to create and test a simple UV spectroscopic method which will be quick, accurate, and precise for estimating quercetin in ophthalmic and other formulations.

Materials and Methods

Instruments

UV/VIS spectrophotometer was used to measure absorbance. All weighing was done on a digital balance and for sonication and degassing of the drug solution bath sonicator was used.

Materials

Quercetin used was purchased from an authentic drug supplier. Other chemicals and solvents used were of analytical grade.

Solvent selection

After assessing the solubility of quercetin in various solvents like distilled water, methanol, and ethanol, it was observed that quercetin is insoluble in distilled water. Quercetin was soluble in ethanol and showed its good spectral characteristics. Therefore, it was selected as a solvent for the development and validation of the UV spectroscopic method.

Stock solution preparation

10 mg drug was dissolved in 100 mL ethanol in a volumetric flask to prepare the stock solution with a concentration of 100 µg/mL of quercetin. Sonication was done for 20 minutes to ensure the proper dissolution of drug in the solvent.

Determination of Lambda Maxima

3 mL of the above prepared stock solution was taken in a test tube and dilution was made by ethanol upto 10 mL to get the concentration of 30 µg/mL. This prepared 10 mL solution was then scanned in the range of 200 to 400 nm by using ethanol as a blank. The maximum wavelength of absorbance of quercetin was found out to be at 255 nm.

Method Validation [16]

Linearity

From the above prepared stock solution, samples were prepared in such a way to achieve the concentrations in the range of 3 to 18 µg/mL. Absorbances were checked at λ max 255 nm, as indicated in Table 2. The same procedure was

repeated 3 times and the amounts of absorbance were recorded. A calibration curve was plotted between concentration vs. absorbance and a straight line was obtained, as displayed in Figure 1.

Table 2. Values of concentration vs. absorbance at lambda max 255 nm

Concentrationsµg/mL	Abs.1	Abs.2	Abs.3	Mean	Standard deviation
3	0.2032	0.2088	0.2056	0.2058	0.00229
6	0.4422	0.4371	0.4070	0.4287	0.01553
9	0.6400	0.6458	0.6413	0.6423	0.00248
12	0.8658	0.9752	0.9398	0.9269	0.04558
15	1.0233	1.3674	1.2104	1.2003	0.14065
18	1.1833	1.9761	1.2269	1.4621	0.36388

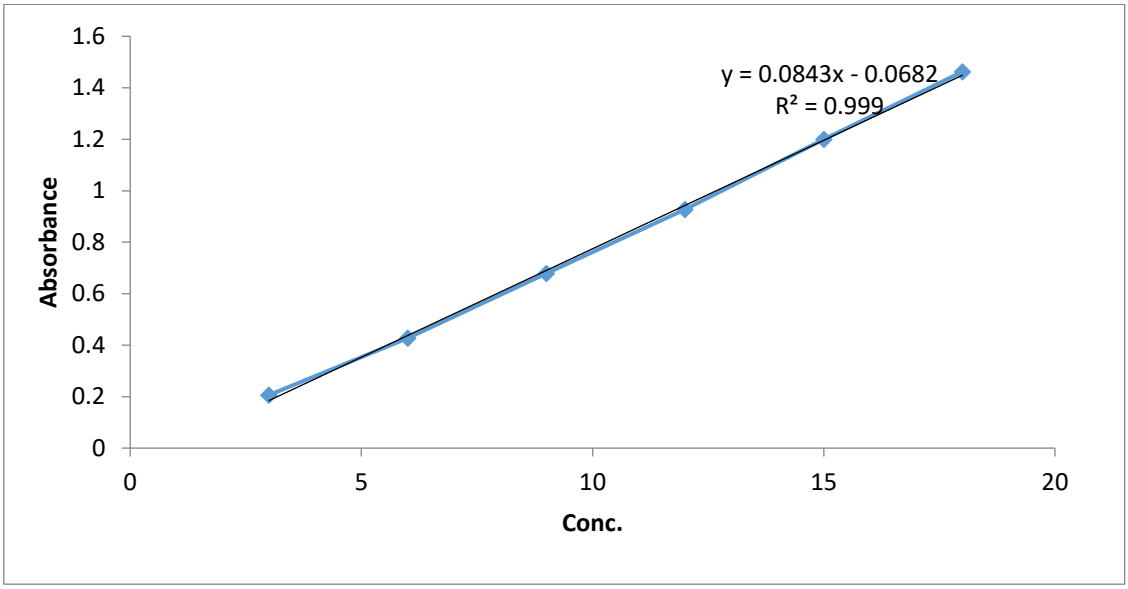


Figure 1. Calibration curve of quercetin in ethanol

Precision

Inter-day and Intra-day precision

To find out whether the proposed method is reproducible or not, the method was analyzed at different time intervals; once at on the same day that is intraday precision, and then on 3 different days inter-day precision. The RSD of their percentage contents was calculated. The RSD percent for intra-day and inter-day assay precision was less than 2.

Accuracy

Recovery studies were carried out as per ICH guidelines to check the accuracy of the proposed method and were carried out at 80, 100, and 120% of the test concentration. The result of the recovery studies are reported in Table 3.

Table 3. Quercetin recovery data

Level	Amount of quercetin added (µg)	Amount of quercetin found (µg)	% Recovery
80%	08	7.77	97.125
100%	10	9.75	97.5
120%	12	11.65	97.08

Ruggedness

Expression of the precision in laboratory variation was carried out by performing the experiment by different analysts. Ruggedness was assessed by different analysts via the same equipment and spike of the standard by 3 times. The results of the same are presented in Table 4.

Table 4. Ruggedness study

Analyst	Amount Taken (µg/mL)	Amount Found (µg/mL ± S.D*)
1	12	11.87
2	16	15.97

Results and Discussion

Linearity range for quercetin was found to be 3-18 µg/mL at 255 nm wavelength. r^2 was found to be 0.999. Good regression values were indicated by quercetin and the recovery studies result reveal that any little variation in the concentration of drug in solutions can be perfectly estimated by this method.

Conclusion

This method is rapid, simple, and economic for the quantitative estimation of quercetin in ocular formulations. Compared with chromatographic methods, this method is accurate and also cost effective.

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