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Isobestic Method for Simultaneous Estimation of Resveratrol and Quercetin

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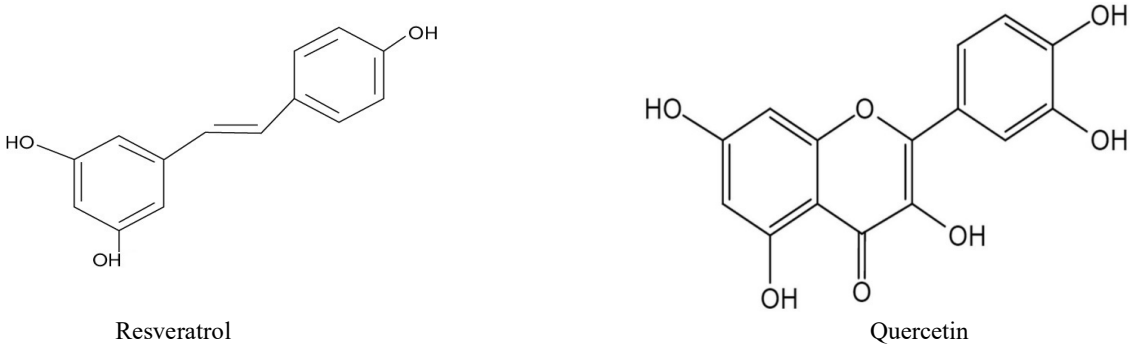
Abstract: *Resveratrol and quercetin are well-known polyphenolic compounds found in everyday foods that have shown tremendous promise in the treatment of a wide range of disorders. For simultaneous estimation of resveratrol and quercetin in their binary combination and marketed formulation, a simple isobestic point method was developed. At 267.6 nm, both drugs were found to have the same absorbance (isobestic point). Resveratrol and quercetin showed linearity ranges of 2-20 mL and 2-25 mL, respectively. For resveratrol, the correlation coefficient was 0.999, while for quercetin, it was 0.998. The resveratrol and quercetin recovery ranges were found to be 98.25 % - 100.85 % and 99.25 % - 100.72 %, respectively, showing method accuracy. The precision and % RSD values were both within accepted limits, indicating method selectivity and repeatability.*

Keywords: Resveratrol, Quercetin, Isobestic Point method, Simultaneous, Estimation, etc.

I. INTRODUCTION

Resveratrol (trans-3, 4, 5-trihydroxystilbene) is a polyphenol found in red wine, rhubarb, blueberries, many red grape types, and peanuts, among other things. Resveratrol (RES) has antioxidant, anti-inflammatory, anticancer, antibacterial, anti-neurodegenerative, and anti-aging properties [1-3]. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is one of the most abundant dietary flavonoids and belongs to the flavonols subgroup. Quercetin has been found to have neuroprotective, gastroprotective, anticarcinogenic, antimicrobial, anti-aging, anti-inflammatory, antioxidative, immunomodulator, antihypertensive, anti-obesity, antihyperglycaemic, lipid regulating, and bone-conserving properties [4-10]. Figure 1 shows the chemical structures of RES and QUE.

According to the literature, numerous analytical methods such as high-performance liquid chromatography (HPLC) [11,12], reverse-phase high performance liquid chromatography (RP-HPLC) [13], and high-performance thin layer chromatography (HPTLC) [14] have been reported for simultaneous estimation of resveratrol and quercetin. However, no spectrophotometric method for simultaneous estimation of RES and QUE utilising the isobestic method has been reported. The goal of this study was to develop a simple, precise, selective, and cost-effective instrumental spectrophotometric method for estimating RES and QUE in tablets.



Resveratrol Quercetin

Figure 1: Chemical Structures of Resveratrol and Quercetin

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II. METHODOLOGY

Instruments:

Schimadzu (UV- 1800) double beam UV-visible spectrophotometer with 1 cm quartz cells and UV-probe 2.34 software connected to a personal computer. Over the wavelength range of 200-400 nm, zero order absorption spectra were observed.

Materials:

The Government College of Pharmacy in Amravati provided the resveratrol and quercetin. Biotrex Neutraceuticals produced resveratrol and quercetin capsules containing 140 mg RES and 100 mg QUE in a 500 mg capsule. The capsules were purchased at a nearby market. Throughout the experiment, analytical grade methanol was used.

Preparation of Standard Solutions:

The standard stock solutions of resveratrol and quercetin were prepared by dissolving precisely weighted 10 mg of each drug in 100 mL methanol to obtain solutions with concentrations of 100 µg/Ml.

Isobestic Point Method

The iso-absorptive method can be used if the spectra of two compounds cross at a certain point. Figure 2 shows the RES and QUE overlay spectra. The overlay spectra show that RES and QUE have maximum wavelengths of 306.4 nm and 255.0 nm, respectively. At 267.6 nm, both drugs were found to have the same absorbance (iso-absorptive point). The wavelengths selected for analysis were 306.4 nm λmax of RES and 267.6 nm iso-absorptive point.

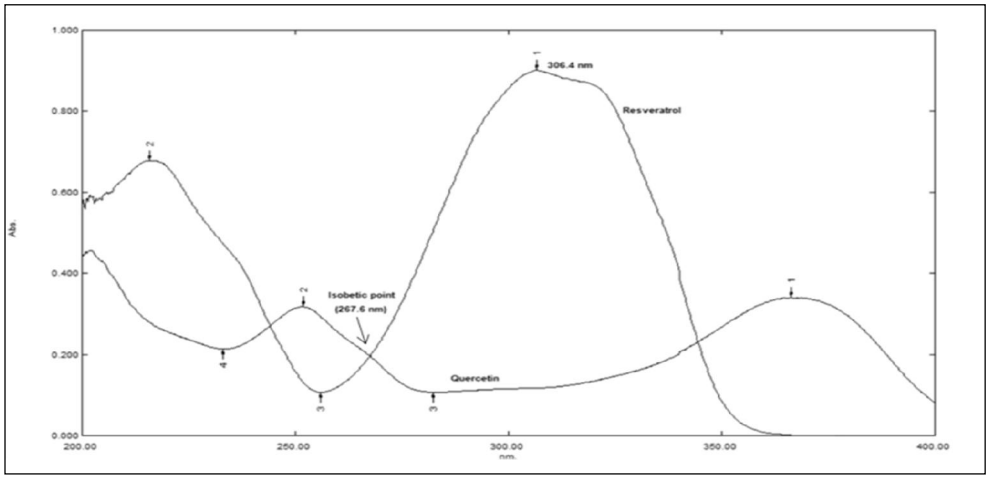

Figure 2: Overlay Spectra of RES (7 µg/mL) and QUE (5 µg/mL)

Application to Standard Laboratory Mixture:

By transferring separate aliquots of each RES and QUE standard solution into a 10 mL volumetric flask, binary mixtures with different ratios of RES and QUE were prepared. Methanol was used to measure absorbances at 306.4 nm and 267.6 nm in a 1 cm cell.

Application to Marketed Formulation:

The average weight was calculated after weighing twenty capsules. The tablets were powdered and thoroughly mixed. A powdered capsule containing 7 mg of RES was accurately weighed and dissolved in 10 mL methanol. The solution was filtered after being sonicated for 15 minutes. To prepare its working solution, which contains 70 µg/mL of RES and 50 µg/mL of QUE, appropriate dilutions of the prepared solution were made.

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III. METHOD VALIDATION

1. Linearity

According to the developed methods, adequate dilutions of standard stock solutions were analyzed for each drug. For RES, the Beer-Lambert concentration range was found to be 2-20 µg/mL while for quercetin, it was found to be 2-35 µg/mL. Table 1 shows the linearity data for the method.

2. Limit of detection

The detection limit is determined by analyzing samples with known analyte concentrations and determining the lowest concentration at which the analyte can be consistently identified.

$LOD = 3.3\times \sigma /S$

Where σ the standard error of y-intercept and S is the slope of the calibration curve.

3. Limit of Quantitation

The quantitation limit is usually determined by analyzing samples with known concentrations and determining the lowest level at which the analyte can be determined with acceptable accuracy and precision. Table 1 shows the result.

$LOQ = 10\times \sigma /S$

Where σ the standard error of y-intercept and S is the slope of the calibration curve.

Table 1: Results of Validation Parameters

Parameters	Resveratrol	Quercetin
Linearity range (µg/mL)	2-20	2-35
Correlation coefficient	0.999	0.998
Slope	0.108	0.011
Standard error of y-intercept	0.01006	0.00638
LOD (µg/mL)	0.307	1.914
LOQ (µg/mL)	0.931	5.8

4. Accuracy

Recovery studies at 80 %, 100 %, and 120 % of the test concentration were carried out according to ICH guidelines to check the accuracy of the proposed method. At each level, the recovery study was repeated three times. Table 2 shows the results of the recovery studies.

Table 2: Recovery Studies

Level of recovery	% Recovery ± SD*	
	RES	QUE
80%	98.64 ± 0.3215	99.25 ± 0.4237
100%	99.86 ± 0.5424	99.86 ± 0.6584
120%	100.85 ± 0.5874	100.72 ± 0.7585

5. Precision:

By assaying the sample solution on the same day and on different days, the intraday and interday precisions were calculated (three replicates). Table 3 summarizes the results.

Table 3: Results of Precision

Days	RES	QUE
Intraday	99.65 ± 0.9854	98.65 ± 0.8547
Interday	100.68 ± 1.2545	99.56 ± 0.5985

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IV. RESULT AND DISCUSSION

The linearity range for QUE and RES was found 2-20 µg/mL and 2-35 µg/mL respectively. The coefficient of correlation for QUE and RES was found 0.999 and 0.998. The good % recoveries were obtained with standard deviation less 2.

V. CONCLUSION

For the simultaneous estimation of resveratrol and quercetin in their laboratory combination and commercial formulation, a new simple spectrophotometric approach was developed. The isobestic method that was developed was precise, cost-effective, and accurate. The developed method could be used to estimate resveratrol and quercetin in their pharmaceutical preparations.

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