Developing a Green UV-Spectroscopic Method for Quercetin and Celecoxib Estimation Using AGREE Assessment Tool

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ABSTRACT

The present study aimed to develop a green UV-spectroscopic method for the estimation of quercetin and celecoxib using the AGREE assessment tool. The simultaneous estimation of quercetin and celecoxib in their original form was achieved by the development and validation of an innovative, straightforward, accurate, repeatable, and efficient simultaneous equation technique. The technique relied on measuring the absorbance of quercetin and celecoxib at 2 wavelengths (373 nm and 251 nm) and determining their λmax. The conc. ranges of 4–12 µg/mL for quercetin and 1.6–4.8 µg/mL for celecoxib were found to have linear calibration curves. It was discovered that the calibration curves for quercetin and celecoxib had linear conc. ranges of 4–12 µg/mL and 1.6–4.8 µg/mL, accordingly, with values for the correlation coefficient (R2) of 0.999. A percentage RSD value was confirmed to be within limits (RSD < 2%) in the precision study. In this work, the greenness of a binary combination of quercetin and celecoxib is measured using spectrophotometric techniques. Within the analytical community, the use of green analytical chemistry has become widely accepted. The analytical GREEnness calculator was used to analyze the assessment's greenness. The simultaneous equation methodology is applied in the technique for quercetin at 373 nm and celecoxib at 251 nm, respectively. The established procedures underwent validation in compliance with ICH recommendations. Quercetin and celecoxib can be successfully estimated together in pharmaceutical and pure dose forms using the suggested method.

Key Words: *Quercetin, Celecoxib, Green analytical chemistry, Simultaneous estimation and UV spectroscopic technique*

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INTRODUCTION

Plant flavonol quercetin belongs to the flavonoid class of polyphenols. 2-(3,4-dihydroxyphenyl) -3,5,7-trihydroxy-4H-chromen-4-one is the chemical name for quercetin. Its potential therapeutic effects in several forms of arthritis, such as osteoarthritis (OA) and rheumatoid arthritis (RA), have been thoroughly researched. The chemical name of Celecoxib 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzene sulfonamideis. Celecoxib is a nonsteroidal anti-inflammatory medication and a COX-2 inhibitor [1]. The FDA recommends celecoxib as a first-line analgesic for patients with osteoarthritis and rheumatoid arthritis. Compared to other NSAIDs like Aspirin, Etodolac, Nabumethone and Salsalate which are prescribed for OA/RA dose of celecoxib is low (100 mg) thus proving better efficiency than other NSAIDs [2]. There aren't many documented UV spectroscopic techniques for figuring out celecoxib and quercetin separately or in various matrices with other medications [3-12]. These methods are selective and sensitive, but they frequently involve the use of dangerous solvents. Therefore, to have a greater positive impact on the environment and workforce, ecologically friendly

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solutions must be developed. Limiting reagent usage, favoring sustainable and minimally hazardous reagents, decreasing waste output as well as minimizing elements that may negatively affect the analyst are all important components of the evaluation of naturalness [13, 14].

Some studies evaluated the greenness analytical GREEnness calculator (AGREE) [15-17]. To determine quercetin and celecoxib in an experimental formulation or synthetic combination selectively, green spectroscopic methods were developed and validated per the recommendations of the ICH [18-20].

The present study aimed to develop a green UV-spectroscopic method for the estimation of quercetin and celecoxib using the AGREE assessment tool.

MATERIALS AND METHODS

Apparatus

UV probe software and a UV 1800 double-beam spectrophotometer were used by Shimadzu. A set of matching quartz cells measuring one centimeter was used to record absorbance values.

Chemicals and reagents

Quercetin as well as celecoxib were kindly provided by Sisco Research Laboratories Pvt. Ltd Taloja Maharashtra, India, and Lupin Pharmaceuticals Pvt. Ltd was used.

Greenness evaluation software

The Analytical GREEnness calculator (AGREE), version 0.5, was used to measure and analyze the method performance's greenness.

Method

EQ approach A calibrated curve for quercetin and celecoxib was created by building the regression equation for each medication and plotting the absorbances at 373 nm and 251 nm, respectively, against the appropriate conc [21].

Assessment methods for green analytical chemistry

Agree

An analytical technique's greenness is indicated by the 12 parameters in the analytical Greenness tool being checked [22] as given in **Figure 1**. This software provides details on reagents, procedures, reagent toxicity, power usage, and other topics [23, 24].

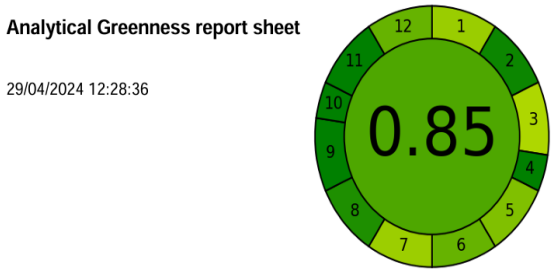
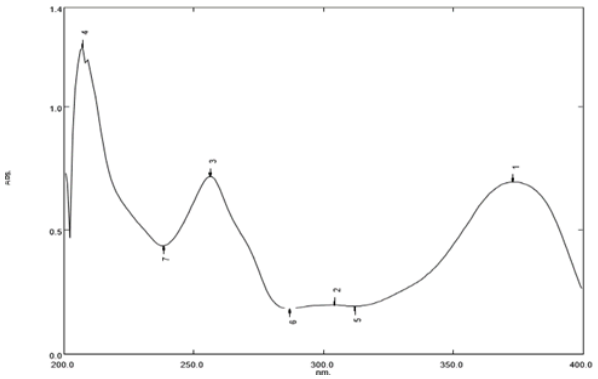


Figure 1. Analytical greenness report sheet

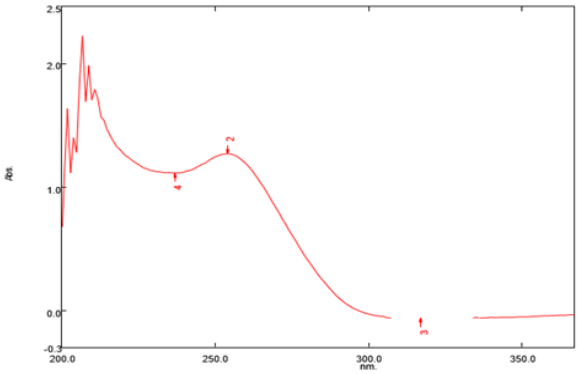
Preparations of standard stock solutions

2.50 mg of quercetin was dissolved in 10 mL of ethanol to create the standard stock solution, then the resulting mixture underwent ten minutes of sonication. For working standard solution with a concentration of 25 µg/mL, 1 mL of the standard stock solution was extracted and diluted with 10 mL of the identical solution. Similarly, 1 mg of celecoxib was dissolved in 10 mL of ethanol to create the standard stock solution. After 10 minutes of sonication, the resultant solution's final volume was adjusted to 10 mL using ethanol. After additional dilution of this solution, a workable standard solution containing 10 mcg/mL was obtained and the graph is given in **Figure 2** [25, 26].

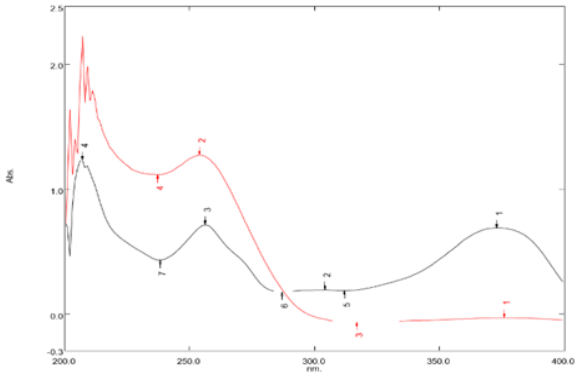
Diagram of overlain spectra



a) UV spectra of quercetin



b) UV spectra of celecoxib



c) Overlay spectra of celecoxib and quercetin

Figure 2. UV spectra of quercetin and celecoxib

Simultaneous equation method development

The 200–400 nm UV range was scanned using the working solutions of both drugs. Both drug's overlay spectra were captured [27]. Using the simultaneous equation approach, wavelengths 373 nm (the λmax of quercetin) and 251 nm (the λmax of celecoxib) were picked from overlain spectra for investigation of both drugs (λ1-373 nm for quercetin and λ2-251 nm for celecoxib) mentioned in **Figure 2** [28]. As a result, it might be able to identify the two drugs using the simultaneous equation approach or the from method [29-32].

Five standard solutions were prepared with a ratio of 2.5:1 (quercetin: celecoxib) with conc. of 4, 6, 8, 10, and 12 mcg/mL for quercetin and 1.6, 2.4, 3.2, 4, and 4.8 µg/mL for celecoxib. Both 373 nm and 251 nm were used to estimate the comparable absorbance [33-35]. Using the SE method and the following formula, the concentrations of drug x (quercetin) and y (celecoxib) in the test solutions have been calculated:

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1} - a x_1 a y_2 \quad (1)$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1} - a x_1 a y_2 \quad (2)$$

The concentrations of quercetin and celecoxib are denoted by Cx and Cy, respectively. At 373 nm and 251 nm, the sample solution's absorbance is measured. Quercetin's absorptivity is measured at 373 nm and 251 nm, while celecoxib's is measured at 373 nm and 251 nm, respectively [36].

Determination of absorptivity value

Table 1 displays the absorptivity values of celecoxib and quercetin from every solution, which were determined using the formula [37].

$$Absorotivity = \frac{Absorbance}{Concentration}(gm/100mL) \quad (3)$$

As directed by ICH [38], the developed approach was validated.

RESULTS AND DISCUSSION

Table 1. Absorptivity value for quercetin and celecoxib

Absorptivity value for quercetin				
Concentration (mcg/mL)	Absorbance	Absorptivity	Absorbance	Absorptivity
	λ_1-373 nm	λ_1-373 nm	λ_2 -251 nm	λ_2 -251 nm
4	0.282	0.0705	0.310	0.0775
6	0.418	0.0696	0.595	0.0991
8	0.473	0.0591	0.645	0.0806
10	0.616	0.0616	0.718	0.0718
12	0.673	0.0560	0.890	0.0741
	Absorptivity for λ_1	0.06336	Absorptivity for λ_2	0.08062
Absorptivity value for celecoxib				
Concentration (µg/mL)	Absorbance	Absorptivity	Absorbance	Absorptivity
	λ_1-373 nm	λ_1-373 nm	λ_2 -251 nm	λ_2 -251 nm
1.6	0.031	0.019	0.202	0.0126
2.4	0.068	0.028	0.416	0.0173
3.2	0.108	0.033	0.719	0.0224
4	0.250	0.0625	0.829	0.0207
4.8	0.420	0.0875	0.978	0.0203
	Absorptivity for λ_1	0.046	Absorptivity for λ_2	0.0187

Agree

The criteria that have been presented align with the twelve SIGNIFICANCE principles, which can be adjusted in weight to offer a certain level of flexibility. Every one of the twelve input variables is converted to a normal 0–1 scale. The ultimate evaluation result is the sum of the

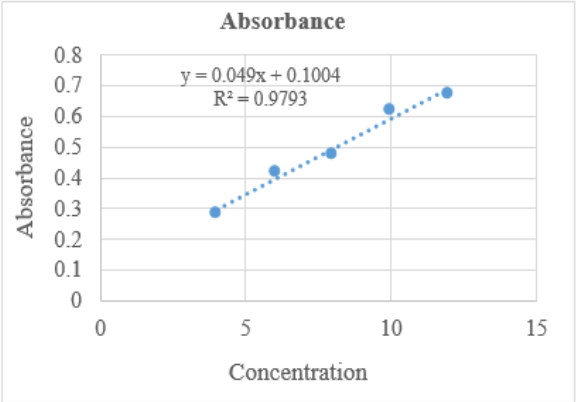
assessment results for each premise. This leads to the creation of a graph that resembles a clock, with the overall score and color representation displayed in the middle [39]. The greenness evaluation report for the experimental procedures is shown and shown in **Figure 1**.

Specificity

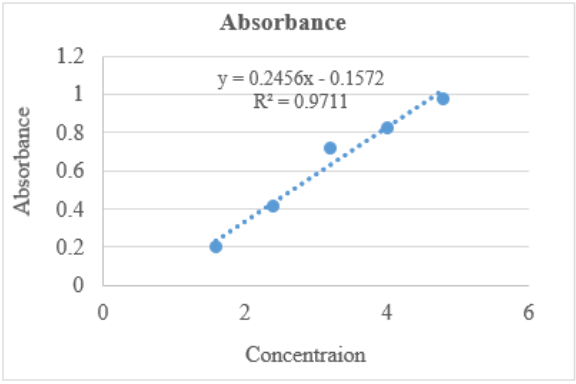
By measuring the absorbance of quercetin and celecoxib separately at 373 nm and 251 nm for the blank and synthesized excipients and comparing their absorbance with the blank and synthesized excipients, the specificity of the procedure was assessed [40-43]. The fact that there was no interference at 251 and 373 nm suggests that the procedure is specific.

Linearity

Graphing the absorbance against the conc. ranges of 4, 6, 8, 10, and 12 mcg/mL for quercetin and 1.6, 2.4, 3.2, 4, and 4.8 mcg/mL for celecoxib allowed for the construction of the calibration curves **(Figure 3)**. In these concentration ranges, the correlation coefficient values of the calibration curves were found to be linear. The sample's conc. and absorbance showed an acute relation, according to the results [44].



a) linearity of quercetin in simultaneous estimation



b) Linearity of celecoxib in simultaneous estimation

Figure 3. Linearity of quercetin and celecoxib in simultaneous estimation

Accuracy

By using the recommended process on simulated excipients containing known levels of each medicine equal to 50%, 75%, 100%, 125%, and 150% of the claims made on the labels of quercetin and celecoxib, the accuracy of the method was assessed label claims [45, 46]. **Table 2** presents the results of calculating the amount of quercetin and celecoxib recovered at each level.

Precision

By evaluating the sample solution's absorbance without altering the test protocol, a precision study was established, and the outcomes are shown in **Table 3** [47].

Repeatability

At least three duplicate measurements of the sample solution's absorbance were made for this investigation on the same day at 0 hours, 8 hours, and 16 hours shown in **Table 3**.

Intermediate precision

By measuring the sample solution's absorbance on three separate days using three distinct equipment and analysts, intermediate precision was achieved shown in **Table 3**.

Reproducibility

Two laboratories verified the method's reproducibility and the outcomes were compared for celecoxib and quercetin shown in **Table 3**. The low percentage of RSD (< 2%) suggested that the procedure is accurate.

Robustness

By varying the wavelength from 373 nm to 251 nm by ± 1 nm, the robustness of the approach was assessed, and the findings are presented in **Table 4**.

Limit of quantification (LOQ) and limit of detection (LOD)

The calibration graph's slope and the response standard deviation were used to calculate LOD and LOQ. The LOD and LOQ of celecoxib were determined to be 13.075 ng/mL and 4.315 ng/mL, respectively, while quercetin was 0.884 ng/mL and 2.681 ng/mL [48, 49].

Stability

For three days at room temperature, the stability of the standard and sample solutions was examined, and daily absorbance measurements were made. Three days pass at room temperature before the sample solution begins to deteriorate, according to the findings of a calculation made on the amount of medication present [50].

Table 2. Recovery results for quercetin and celecoxib

Concentration (%)	Quercetin				Celecoxib			
Quercetin/ Celecoxib	Amt added (mg)	Recovered amt (mg)	Recovered amt (%)	RSD (%)	Amt added (mg)	Recovered amt (mg)	Recovered amt (%)	RSD (%)
50	1.94	1.95	100.51 ± 0.32	0.32	0.72	0.71	98.61 ± 0.76	0.76
75	2.19	2.21	100.91 ± 0.17	0.17	0.89	0.81	100.09 ± 0.51	0.51
100	2.50	2.52	100.8 ± 0.15	0.15	1.0	0.9	99.50 ± 0.38	0.38
125	2.69	2.68	99.62 ± 0.18	0.18	1.90	1.86	99.15 ± 0.61	0.61
150	2.88	2.91	100.69 ± 0.07	0.07	2.21	2.11	98.47 ± 0.50	0.50

The range of percentage recovery for quercetin and celecoxib was 99.62 to 100.51 and 98.47 to 100.09, respectively, suggesting that the method's accuracy was satisfactory.

Table 3. Precision result for quercetin and celecoxib

Specifications	Time of sampling	Quercetın			Celecoxıb		
		Amt present (mg)	Amt present (%)	RSD (%)	Amt present (mg)	Amt present (%)	RSD (%)
Repeatability (n = 30)	0 hrs	1.9	100.46 ± 0.87	0.48	0.6	100.88 ± 0.98	0.97
	8th hrs	2.3	100.46 ± 0.49	0.46	0.7	100.86 ± 0.20	1.20
	16th hrs	2.2	100.46 ± 0.25	0.25	0.8	100.08 ± 0.76	0.36
Intermediate precision (n = 6)	1st day	2.10	100.93 ± 0.10	0.46	0.7	100.62 ± 0.75	0.74
	2nd day	2.35	100.52 ± 0.12	0.51	0.6	100.14 ± 0.59	0.58
	3rd day	1.90	100.10 ± 0.17	0.12	0.8	100.88 ± 0.29	0.30
	Analyst-1	2.30	100.12 ± 0.33	0.33	0.9	100.51 ± 0.26	0.46
	Analyst-2	2.25	100.24 ± 0.20	0.03	0.7	100.12 ± 0.98	0.58
	Instrument-1	2.20	100. 34 ± 0.51	0.22	0.8	100.62 ± 0.46	0.97
	Instrument-2	2.22	100. 17 ± 0.25	0.25	0.6	100.34 ± 0.98	0.37
Reproducibility (n = 6)	Lab-1	2.34	100.12 ± 0.20 100.34 ± 0.12	0.12 0.20	0.6 0.8	100.23 ± 0.20 100.66 ± 0.38	0.36 0.37
	Lab-2	2.22	100.12 ± 0.20 100.34 ± 0.12	0.12 0.20	0.6 0.8	100.23 ± 0.20 100.66 ± 0.38	0.36 0.37

The method's precision was indicated by the low percentage RSD (< 2%) for both celecoxib and quercetin.

Table 4. The result was observed by changing the wavelength ± 1 nm.

Quercetin				Celecoxib			
Wavelength (nm)	Amt present (mg)	Amt present (%)	RSD (%)	Wavelength (nm)	Amt present (mg)	Amt present (%)	RSD (%)
372	2.35	99.87±0.10	0.67	250	0.89	99.67±0.10	0.70
374	2.27	100 ± 0.24	0.45	252	0.86	99.23±0.24	0.39

The computed percent RSD value for PAR and FLU in the robustness investigation was less than 2%, indicating the robustness of the approach.

CONCLUSION

The simultaneous equation method that was created is straightforward, accurate, precise, and specific. The simultaneous estimate of quercetin and celecoxib in pure and pharmaceutical dosage forms without interference from excipients was demonstrated to be a reliable and selective process through statistical analysis. These medications can be routinely estimated using this new, straightforward method. To assess quercetin and celecoxib concurrently in their pure forms, synthetic mixtures, and dosage forms created in laboratories with varying ratios of active ingredients, new, accurate, and dependable spectrophotometric techniques have been developed. Through the use of AGREE, the ecological impact of the techniques was investigated, and it was concluded that the

suggested processes are environmentally beneficial. The fact that these methods are simple and don't require expensive or complex equipment is another advantage.

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Conflict of interest: None

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Ethics statement: None

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