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Research Article

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Determination of quercetin by UV spectroscopy as quality control parameter in herbal plant: *Cocculus hirsutus*

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ABSTRACT

Quercetin is polyphenol flavonoid and exhibits anti-inflammatory, antihepatotoxic antihepatotoxic, antiulcer, antiallergic, antidiabetic, antiviral and antioxidant activity. It is found in many herbal plants such as in Cocculus hirsutus. The aim of this research work is to develop a simple, accurate, precise and economic UV spectroscopic method for the determination of quercetin. The estimation of quercetin by spectroscopic with maximum absorption at λmax 256.30 nm using developed solvent [n-butanol : water : acetic acid (7:1:1)]. Beer-Lambert's law is obeyed in the concentration range of 0-12μg/ml and is described by the regression equation y = 0.063x + 0.005 with a regression coefficient (r^2) = 0.999 (n = 5). For Quercetin, the value of molar absorptivity and Sandell's sensitivity are 6.3197 x10^2 L/mol/cm and0.4782 μg/cm^2, respectively and of LOD and LOQ are found to be 0.4486 and 1.3595μg/ml, respectively. The percentage recovery of quercetin was found to be 98.66 %. The % RSD for intraday and interday precision was 1.27 and 1.40 respectively showed excellent % RSD which is less than 2. The developed method was validated in terms accuracy, precision, linearity and robustness. The statistically validated results indicate that the proposed method has good sensitivity, accuracy and precision. The method is simple and economic as compare to chromatographic methods. The developed method was successfully applied for the determination of Quercetin in herbal plants and its product.

Keywords: Quercetin; UV Spectroscopy; Validation; *Cocculus hirsutus*.

INTRODUCTION

Flavonoids are a large class of natural polyphenol compounds of low molecular mass, widely distributed in plant world, where they perform several very important functions such as antioxidant and chelating properties [1-3]. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies., including anti-oxidation, anti-allergic[4], anti inflammatory[4-5], antioxidant[5], antimicrobial (antibacterial [6-7] antifungal and antiviral[8-9], anti-cancer[10] and anti-diarrheal activities.[11]. In the human diet, they are most concentrated in fruits, vegetables, wines, teas and cocoa.

As they probably behave in plants as photoprotectors, besides the systemic actions exploited by flavonoids ingested with vegetables or food supplements, it has been suggested that they can be used as sunscreens [12]. To be efficient in its photoprotective action, a sunscreen must have an absorption spectrum wide enough to cover as many of the wavelengths of the UV region as possible.

Quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavone, **Figure 1**) is one of the most widespread compounds of the natural flavonoids class.

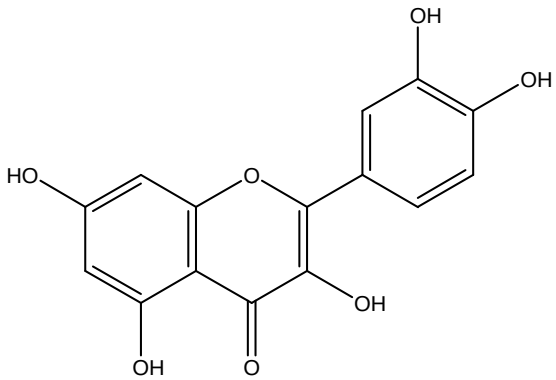

Figure 1: Structure of Quercetin

Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques.

The literature revealed that a few numbers of papers were reported or directed toward the detection of quercetin in plants by UV-spectrophotometric method[13-14]. As per author's best knowledge yet there is no spectroscopic method is available for the estimation of quercetin in the leaves of *Cocculus hirsutus* [15]. The aim of this research is to reduce analysis time for the determination of quercetin in the leaves of *Cocculus hirsutus* by UV spectroscopy.

EXPERIMENTAL SECTION

Instruments

Absorbance measurements was made on Shimadzu 1800 double beam UV/VIS spectrophotometer provided with a pair of matched quartz cells of 1 cm width, Shimadzu digital balance used for weighing, and Ultra sonicator of PCI Analytics instruments was used sonicating the drug and sample solution.

Materials

Plant Material was collected from Aurangabad district, Maharashtra, India. It was authenticated at Botanical Department of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. Its authentication number is BOT/2012-13/0552. The ethanol extract of air shade dried material of *C. hirsutus* leaves was taken by hot soxhlet extraction technique for experiment. Flavanoids standards (Quercetin) purchased from Natural remedies, Bangalore, India (purity >97%). All the chemicals and reagents were of analytical grade and were purchased from S.D fine, Mumbai.

Selection of common solvent

After assessing the solubility of quercetin in different solvents, developed solvent [n-butanol : water : acetic acid (7:1:1)] shows good spectral characteristics.

Preparation of standard stock solution

The standard stock solutions of quercetin was prepared by dissolving 100 mg of each drug in developed solvent [n-butanol : water : acetic acid (7:1:1)] and final volume was adjusted with same solvent in 100 ml of volumetric flask to get a solution containing 1000 µg/ml of quercetin (Stock A).

Selection of wavelength

In a 10 ml volumetric flask, pipette out 1ml standard stock A solution of quercetin and dilute it up to the mark with the developed solvent[n-butanol : water : acetic acid (7:1:1)] to get a concentration of 100µg/ml (Stock B). The stock B solution of quercetin and ethanolic extract of *Cocculus hirsutus* were scanned between 200 to 400 nm and 256.30 nm was found to be maximum wavelength for absorption as shown in **Figure 2** and **Figure 3** respectively. This wavelength was selected for development of UV method for estimation of quercetin in ethanolic extract of *Cocculus hirsutus*.

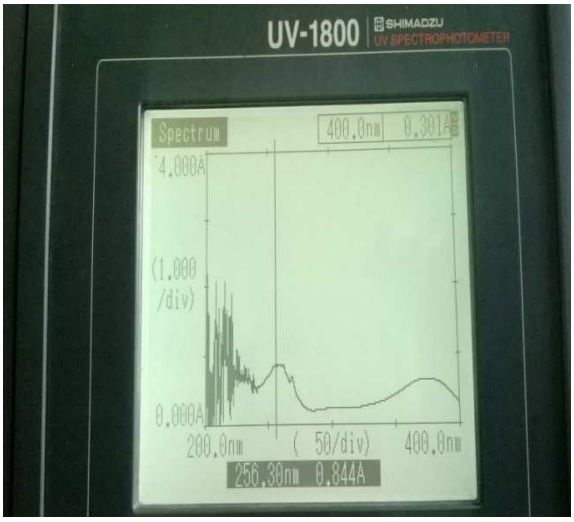

Figure 2: Spectrum of Standard Quercetin

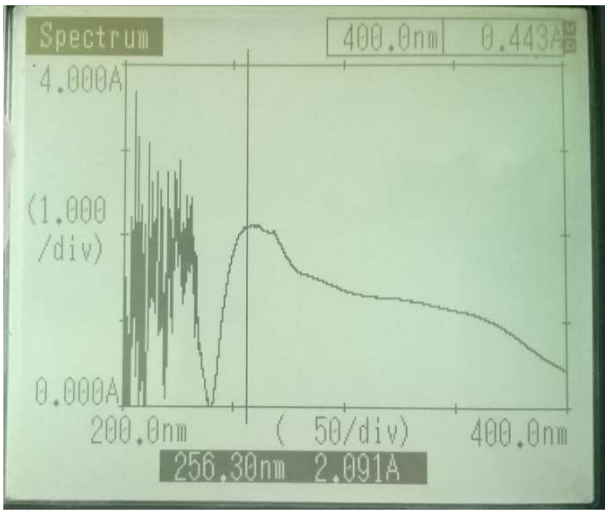

Figure 3: Spectrum of Ethanolic Extract of *Cocculus hirsutus*

Method Validation [16]:

Linearity

The standard stock solutions of quercetin (Stock A) was prepared by dissolving 100 mg of each drug in developed solvent [n-butanol : water : acetic acid (7:1:1)] and final volume was adjusted with same solvent in 100 ml of volumetric flask to get a solution containing 1000 µg/ml of quercetin. Aliquots of working stock solutions of quercetin were prepared with developed solvent to get concentration in range of 0-12 µg/ml. The absorbance of resulting solutions were measured at λ max 256.30 nm and reported in **Table 1**. A calibration curve as concentration vs. absorbance was constructed to study the Beer-Lambert's Law and the regression equation (**Figure 4**).

Table 1: Quercetin in *Cocculus hirsutus*

Concentration(µg/ml)	Absorbance
0	0.000
2	0.141
4	0.256
6	0.391
8	0.51
10	0.656
12	0.763

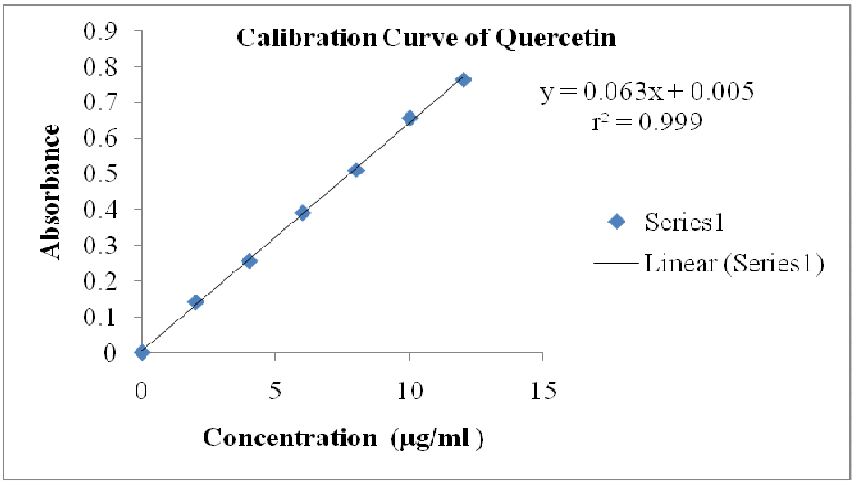

Figure 4: Calibration curve of Quercetin

Precision:

Interday and Intraday precision

The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively (six replicat**es).** The results of the same are presented in **Table 2**.

Table 2: Evaluation of Intraday and Interday Accuracy and Precision for Quercetin

QCT taken (µg/ml)	Intraday Accuracy and precision			Interday Accuracy and precision		
	QCT found (µg/ml)	RE %	RSD %	QCT found (µg/ml)	RE %	RSD %
10	10.53	0.0561	1.3052	10.26	0.049	1.1911
15	15.23	0.0861	1.3829	15.55	0.101	1.5914
20	20.51	0.0945	1.1291	19.85	0.117	1.4435

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The result of the recovery studies are reported in **Table 3**.

Table 3 : Recovery data of Quercetin.

Level	Amount of QCT added (µg)	Amount of QCT found (µg)	% Recovery	% RSD*
80 %	08	7.82	97.75	1.72
100 %	10	9.95	99.50	1.57
120 %	12	11.85	98.75	1.63

**An average value ± relative standard deviation of 5 observations*

Ruggedness

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times with different analyst by using same equipment. The results of the same are presented in **Table 4.**

Table 4: Ruggedness study

	Amount taken of Quercetin(µg/ml)	Amount Found of Quercetin (µg/ml ± S.D*)
Analyst 1	10	9.89
Analyst 2	10	9.87

Limit of detection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$DL = \frac{3.3\sigma}{S}$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

Limit of quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$QL = \frac{10\sigma}{S}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve

Analysis of the Ethanolic Extract of *Cocculus hirsutus*

A quantity of Ethanolic Extract of *Cocculus hirsutus* 50 mg was transferred to 50 ml volumetric flask and dissolved in developed solvent and final volume was made up with same solvent. The sample solution was then filtered through Whatman filter paper No.41. From the above solution 0.373ml of solution was taken and diluted to 10 ml with methanol to get final concentration containing 37.33µg of solution containing 51.6666 µg/ml of quercetin. Analysis procedure was repeated six times with Ethanolic Extract of *Cocculus hirsutus*. The results for Ethanolic Extract of *Cocculus hirsutus* analysis are reported in **Table 5**.

Table 5: Result of analysis of Ethanolic Extract of *Cocculus hirsutus*

Formulation	Drug	**Percentage of QCT ± S.D***
50 mg Ethanolic Extract of *Cocculus hirsutus*	Quercetin (6.925 mg)	13.85 ±0.44

RESULTS AND DISCUSSION

Linearity range for quercetin is 0-12 µg/ml at wavelength 256.30 nm. The coefficient of correlation for quercetin is 0.999. Quercetin shows good regression value and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of Quercetin in Ethanolic Extract of *Cocculus hirsutus* found by method is 13.85 ±0.44. The validation parameters of quercetin by uv spectroscopic method is summarized in **Table 6**.

Table 6: Validation parameters of Quercetin for UV-Spectroscopic method

Parameter	**Analytical data**
Linearity Range (µg/ml)	0-12
λ max (nm)	256.30
Molar extinction coefficient, L/mol/cm	6.3197 x10^2
Sandell's sensitivity, µg/cm^2	0.4782
Slope	0.063
Intercept	0.005
Standard deviation about regression (Sy)	±0.0100
Standard deviation of Slope (Sb)	±0.0009
Standard deviation of intercept (Sa)	±0.0068
Correlation co-efficient (r)	0.999
Limit of detection (LOD, µg/ml)	0.4486
Limit of quantification (LOQ, µg/ml)	1.3595
Intraday Precision (% RSD)	1.2724
Interday Precision (% RSD)	1.4086
Accuracy (% RSD)	1.64
Accuracy (% Recovery)	98.6666

CONCLUSION

The proposed uv spectroscopic method is precise and cost effective as compare to chromatographic methods. The simplicity, rapidity and economy of uv spectroscopy method for determination of quercetin in leaves of *Cocculus hirsutus* makes it suitable as a quality control parameter in herbal plants.

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