



## Research Article

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## DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPY FOR THE ESTIMATION OF RUTIN FROM ETHANOLIC EXTRACT OF *COCCULUS HIRSUTUS* LEAVES

Patil Vandana P.<sup>\*1</sup>, Angadi Sachidanand S.<sup>2</sup> and Devdhe Subhash J.<sup>2</sup><sup>1</sup>Department of Pharmaceutical Analysis, Yash Institute of Pharmacy, Aurangabad, Maharashtra, India<sup>2</sup>Department of Pharmacology, Yash Institute of Pharmacy, Aurangabad Maharashtra, India

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**\*Corresponding author**Prof. Vandana P. Patil, Yash Institute of Pharmacy, Aurangabad-431134 Maharashtra, India  
E-mail: vandana2609@gmail.com

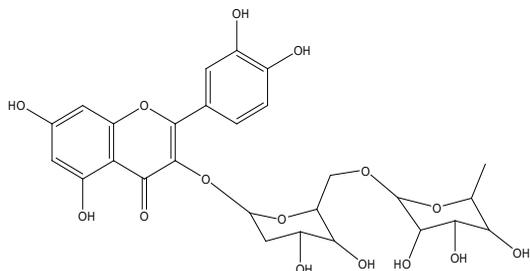
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**ABSTRACT**

Rutin is a citrus flavonoid glycoside and exhibits anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral and antioxidant activity. It is found in many herbal plants. The aim of this research work was to develop and validate a simple, accurate, precise and economic UV spectroscopic method for the determination of rutin in *Cocculus hirsutus*. The estimation of rutin by spectroscopic with maximum absorption at  $\lambda_{max}$  262.10 nm using developed solvent [n-butanol : water : acetic acid (7:1:1)]. Beer-Lambert's law was obeyed in the concentration range of 0-18  $\mu\text{g/ml}$  and was described by the regression equation  $y = 0.032x + 0.008$  with a regression coefficient ( $r^2$ ) = 0.999 (n = 5). For Rutin, the value of molar absorptivity and Sandell's sensitivity were  $1.6429 \times 10^6$  L/mol/cm and  $0.0372 \times 10^2$   $\mu\text{g/cm}^2$ , respectively and of LOD and LOQ were found to be 0.6135 and 1.8593  $\mu\text{g/ml}$ , respectively. The percentage recovery of rutin was found to be 98.22 %. The % RSD for intraday and interday precision was 1.33 and 1.52 respectively. It 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 Rutin in herbal plants and its product.

**Keywords:** Rutin; UV Spectroscopy; Validation; *Cocculus hirsutus*.**INTRODUCTION**

Flavonoids are a large class of natural polyphenol compounds and have been shown to have a wide range of biological and pharmacological activities. In the human diet, they are most concentrated in fruits, vegetables, wines, teas and cocoa<sup>1</sup>. Rutin (RUT) is a citrus flavonoid glycoside. Chemically it is 5, 7, 3, 4, tetrahydroxy flavonol -3-rhamanoglucoside (Figure 1) and widely used in medicine for maintenance of capillary integrity. It possesses anti-inflammatory, antihepatotoxic<sup>2</sup>, antiulcer<sup>3</sup>, antiallergic and antiviral actions and some of them provide protection against cardiovascular mortality<sup>4-6</sup>. It also has antioxidant activity and reduces low density lipoproteins [LDL] oxidation<sup>7</sup>.

**Figure 1: Structure of Rutin (RUT)**

Natural products are an important source of new compounds leading to drugs in all major disease areas.

They represent a pool of structures that have been the source of most of the active ingredients of medicines. *Cocculus hirsutus* is one of the important medicinal plant. *Cocculus hirsutus* is a perennial climbing scandent shrub with hairy sepals belonging to family Menispermaceae, found in Sudan, central Asia, China and India (throughout tropical and subtropical regions). Almost all parts of the plant are helpful to treat different kinds of pharmacological activity such as Larvicidal, Anti-ulcer, Hepatoprotective, Antihyperglycemic, Antioxidant, Antimicrobial, Antipyretic, Anthelmintic, Anti-arthritis, Anti-fertility, Anti-tumour, Diuretic, Laxative, Anti-convulsant, Antiinflammatory and Analgesic activity<sup>8</sup>.

The literature revealed that a few numbers of papers were reported toward the detection of rutin in plants by UV-spectrophotometric method<sup>9-11</sup>. As per author's best knowledge yet there is no spectroscopic method available for the estimation of rutin in the leaves of *Cocculus hirsutus*. The aim of this research was to reduce analysis time for the determination of rutin in the leaves of *Cocculus hirsutus* by UV spectroscopy.

**MATERIALS AND METHODS****Instruments**

Shimadzu 1800 double beam UV/VIS spectrophotometer provided with a pair of matched quartz cells of 1 cm width was used for absorbance measurement. Shimadzu digital balance was used for weighing. 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 ethanolic extract of air shade dried material of *Cocculus hirsutus* leaves was taken by hot soxhlet extraction technique for experiment. Flavonoids standards (Rutin) purchased from Natural remedies, Bangalore, India (purity >97%). All the chemicals and reagents were of analytical grade and were purchased from S.D. fine chemicals, Mumbai, India.

**Selection of common solvent**

After assessing the solubility of rutin in different solvents, we found solvent comprising of n-butanol : water : acetic acid (7:1:1)] showed good spectral characteristics.

**Preparation of standard stock solution**

Accurately weigh and dissolve 100 mg of standard rutin 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 rutin (Stock A).

**Selection of wavelength**

In a 10 ml volumetric flask, pipette out 1ml standard stock A solution of rutin 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 rutin and ethanolic extract of *Cocculus hirsutus* were scanned between 200 to 400 nm and 262.10 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 rutin in ethanolic extract of *Cocculus hirsutus*.

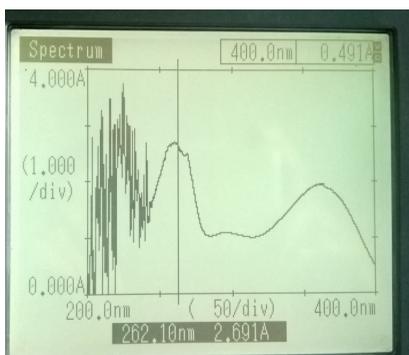


Figure 2: Spectrum of Standard Rutin

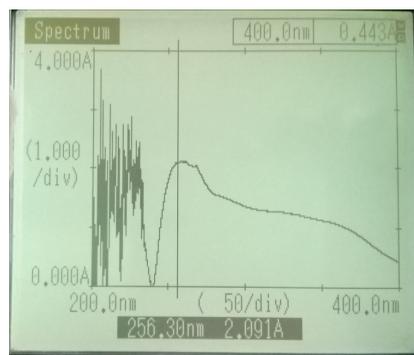


Figure 3: Spectrum of Ethanolic Extract of *Cocculus hirsutus*

**METHOD VALIDATION**

**Linearity**

The standard stock solutions of rutin (Stock A) was prepared by dissolving 100 mg of standard rutin 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 rutin. Aliquots of working stock solutions of rutin were prepared with developed solvent to get concentration in range of 0-18 µg/ml. The absorbance of resulting solutions were measured at λ max 262.10 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)<sup>12</sup>.

Table 1: Rutin in *Cocculus hirsutus*

Concentration(µg/ml)	Absorbance
0	0
2	0.075
6	0.208
10	0.332
14	0.458
18	0.583

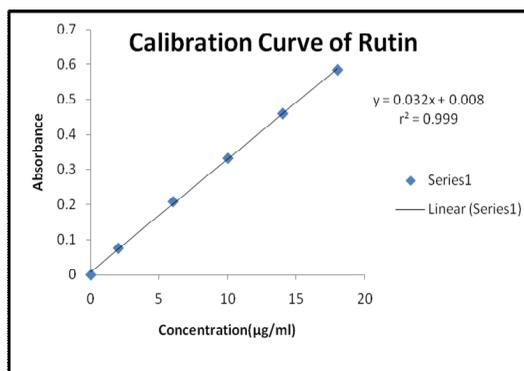


Figure 4: Calibration curve of Rutin

**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

replicates). The results of the same are presented in Table 2.

**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.

**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.

**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  $\sigma$  = 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  $\sigma$  = the standard deviation of the response  
S = the slope of the calibration curve

**Table 2: Evaluation of Intraday and Interday Accuracy and Precision for Rutin**

RUT taken (µg/ml)	Intraday Accuracy and Precision			Interday Accuracy and Precision		
	RUT found (µg/ml)	RE %	RSD %	RUT found (µg/ml)	RE %	RSD %
10	10.14	0.0646	1.5616	10.23	0.0689	1.6508
15	15.06	0.0659	1.0712	15.11	0.0768	1.2454
20	20.82	0.1168	1.3741	20.24	0.1394	1.6871

**Table 3: Recovery data of Rutin**

Level	Amount of RUT added (µg)	Amount of RUT found (µg)	% Recovery	% RSD*
80%	8	7.78	97.25	1.73
100%	10	9.85	98.5	1.66
120%	12	11.87	98.91	1.57

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

**Table 4: Ruggedness study of Rutin**

	Amount taken of RUT (µg/ml)	Amount found of RUT (µg/ml ± S.D*)
Analyst 1	10	9.85
Analyst 2	10	9.86

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

Formulation	Drug	Percentage of RUT ± S.D*
50 mg Ethanolic Extract of <i>Cocculus hirsutus</i>	Rutin (5.6339 mg)	11.26 ± 0.54

**Table 6: Validation parameters of Rutin for UV-Spectroscopic method**

Parameter	Analytical data
Linearity Range (µg/ml)	0-18
$\lambda$ max (nm)	262.10
Molar extinction coefficient, L/mol/cm	$1.6429 \times 10^6$
Sandell's sensitivity, µg/cm <sup>2</sup>	$0.0372 \times 10^{-2}$
Slope	0.032
Intercept	0.008
Standard deviation about regression (Sy)	±0.0129
Standard deviation of Slope (Sb)	±0.0008
Standard deviation of intercept (Sa)	±0.0087
Correlation co-efficient (r)	0.999
Limit of detection (LOD, µg/ml)	0.6135
Limit of quantification (LOQ, µg/ml)	1.8593
Intraday Precision (% RSD)	1.3356
Interday Precision (% RSD)	1.5277
Accuracy (% RSD)	1.6533
Accuracy (% Recovery)	98.22
Assay of rutin in ethanolic extract of <i>Cocculus hirsutus</i> (%)	11.26 ± 0.54

### 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 0.373 ml of solution was taken and diluted to 10 ml with methanol containing 42.0634 µg/ml of rutin. 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.

### RESULTS AND DISCUSSION

Linearity range for rutin was 0-18 µg/ml at wavelength 262.10 nm. The coefficient of correlation for rutin was 0.999. Rutin 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 Rutin in ethanolic extract of *Cocculus hirsutus* found by method was 11.26 ±0.54. The validation parameters of rutin by uv spectroscopic method is summarized in Table 6.

### 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 rutin in leaves of *Cocculus hirsutus* makes it suitable for the estimation of rutin in herbal plants.

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