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UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF RUTIN

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Key words: Rutin, UV spectrophotometric method, Validation.

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ABSTRACT

***Plan*:** *we have developed new a method for quantitative estimation of Rutin hence no UV spectroscopic method is available for the quantitative analysis of rutin for bulk analysis.*

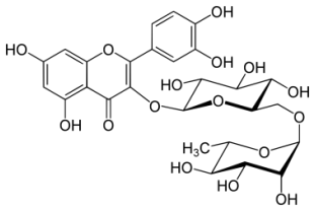
***Preface*:** *U.V Spectrophotometric method has been widely employed for determination of analyte in a mixture. Our aim is to develop spectroscopic method for estimation of the Rutin m in ternary mixture by using U.V spectrophotometry.*

***Methodology*:** *The method was validated as per ICH guidelines. The recovery studies confirmed the accuracy and precision of the method.*

***Outcome*:** *It was successfully applied for the analysis of the drug in bulk and could be effectively used for the routine analysis.*

1. INTRODUCTION

Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonoid compound. Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose (α-L Rhamnopyranosyl-(1→6)-β-D-glucopyranose). It is a flavonoid of the flavonol type that occurs plentifully in *S. Involucrata*. Previous studies have reported that rutin displays several pharmacological properties, including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombotic, vasoprotective, cardioprotective, and neuroprotective activities[1, 2].



Rutin

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2. MATERIAL AND METHOD

2.1. Material

The rutin gift sample was obtained from Loba chem. Pvt. Ltd (Mumbai, India) and used as the working standard.

2.2. Instrumentation

A double beam UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) connected to computer loaded withspectra manager software UV Probewas used. The spectra were obtained withthe instrumental parameters as follows: Wavelengthrange: 200–400 nm. All weights were taken on anelectronic balance (Model Shimadzu AUX 120).

2.3. Preparation of standard stock solution

Specified quantity of rutin (10 mg) was dissolve in 100 ml of methanol (100 µg/mL). Out of this stock 0.2-1.2 ml was pipetted and diluted up to 10 ml by methanol (2-12 µg/mL) and examined between 200-400 nm. The maximum absorbance was determined using UV-Vis Specrophotometer (UV-1700, Shimadzu, Japan) to confirm the λmax of the drugs.

2.4. Validation of analytical method

The analytical performance characteristics which may be tested during methods validation: % Recovery, Precision, Ruggedness and sensitivity[3, 4, 5].

3. RESULTS AND DISCUSSION

3.1. Method Development

The solution of rutin in methanol was found to exhibit maximum absorption at 257 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and the procured drug sample of rutincomplies with the reference spectra (Figure 1).

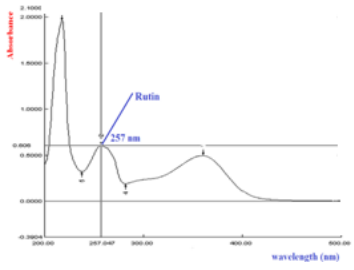

Figure 1. UV spectra of Rutin.

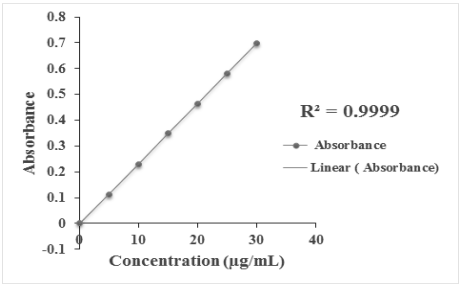

Figure 2. Calibration curve of Rutin

3.2. Validation of analytical method

3.2.1. Linearity

Accurately weighted rutin(10 mg) was dissolved in 100 ml of methanol to obtain working standard of 100 µg/ml. Aliquots were pipetted from the stock solution of drug and were transferred to 10 ml volumetric flask, the final volume was adjusted with methanol so that concentration of 2-12 µg/ml could be made. Absorbance of the above solution were taken at 257 nm by using UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan) against the blank solution prepared in the same manner without adding the drug. A graph of absorbance vs concentration was plotted (Figure 2) and R^2 was found to be 0.9999.

3.2.2. Recovery

Recovery study is performed by standard addition method by adding the known amount of rutin(Working standard) at two different concentration levels i.e 80%, 100% of assay concentration and % recovery for all these drug were calculated. Result was reported in Table 1.

Table 1: Recovery study

Drug	*Initial amount (µg/ml)*	*Added Amount (µg/ml)*	*% Recovery*	*% RSD (n = 3)*
Rutin	2	2.0	100.15	0.05
	2	1.9	100.98	0.02

3.2.3. Precision

Intra-day precision was determined by analysing, the two different concentrations 2 mg/ml, 3 mg/ml containing rutin, for three times in the same day (n = 3) Table 2. Inter-day variability was assessed using above mentioned three concentrations analysed on three different days, over a period of one week (n = 3) Table 2.

Table 2: Presion study

		Intra - Day		*Inter - Day*	
Drug	*Con. (µg/ml)*	*Mean ± SD*	*% RSD*	*Mean ± SD*	*% RSD*
Rutin	2	2.0 ± 0.0016	0.05	2.0 ± 0.0014	0.05
	3	3.0 ± 0.0014	0.02	2.9 ± 0.0048	0.01

3.2.4. Ruggedness

From stock solution, sample solution containing rutin (2 µg/ml) was prepared and analyzed by two different analysts using similar operational and environmental conditions (Table 3) (n = 3).

Table 3: Ruggedness study

	% Amount Found		*% RSD*	
Drug	Analyst I	Analyst II	Analyst I	Analyst II
Rutin	100.44	100.89	0.01	0.03

3.2.5. Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ) (Table 4).

Table 4: Sensitivity study

Drug	*LOD*	*LOQ*
Rutin	0.29 ± 0.002	0.88 ± 0.016

4. CONCLUSION

The proposed UV spectrophotometric method was found very simple, rapid and economical. The method is validated in compliance with ICH guidelines is suitable for estimation of rutinwith excellent recovery, precision and linearity.

5. ACKNOWLEDGEMENTS

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