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Research Paper

Development and validation of UV spectrophotometric method for simultaneous estimation of Rutin and Quercetin novel gel formulation and analysis of wavelength in buffer at pH 7.4

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Abstract:
The aim of this study is to authenticate a proposed simultaneous methodology for quercetin and rutin by UV-visible spectroscopy technique. In present time, research come up with various combination formulations and its significance increased day by day. These combination formulation are having great importance in providing treatment with less side effects and faster action to meet standard criteria. To validate their therapeutic effect different analytical methods performed. These analytical techniques provides statistical validation. As rutin and quercetin shows maximum absorbance at 264 and 371nm respectively. Both the drugs, Rutin and Quercetin obey the Beer- lambert's law in the concentration ranges of 0-12µg/ml. Simultaneous method for both the drugs validate with UV- spectrophotometer to determine single wavelength.

Keywords: *Rutin, Quercetin, Simultaneous method, validation, U.V. spectrophotometer*

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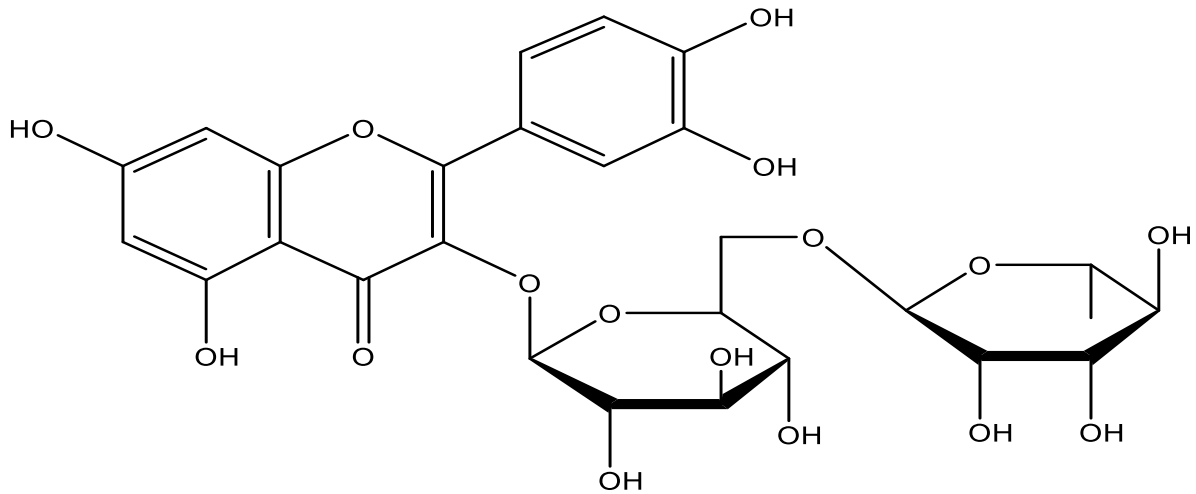
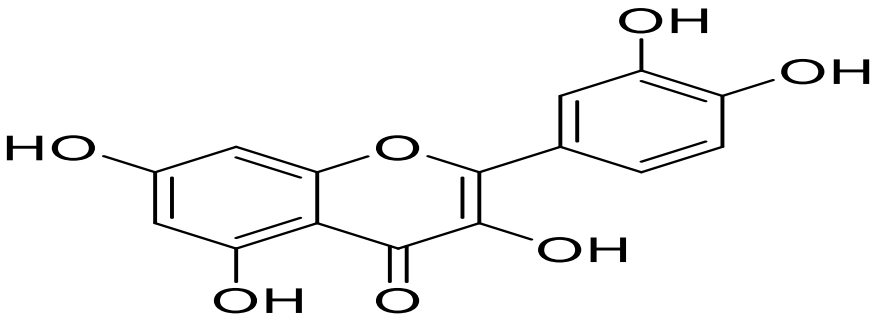
I. Introduction:

Flavonoids, a class of phenolic compounds whose name originates from the Latin term "flavus," denoting yellow, exhibit a broad spectrum of biological activities. These include antiallergenic, antiviral, anti-inflammatory, and vasodilating effects. Quercetin is a therapeutic agent commonly used to treat various allergic conditions, including hives, eczema, and asthma. Its applications also extend to cardiovascular health, addressing issues such as atherosclerosis, high cholesterol, and circulation problems. Additionally, quercetin has been used to manage chronic fatigue syndrome, cancer, and prostate infections.

Rutin



Quercetin



Quercetin, a flavonol compound (3,3',4',5,7-pentahydroxyflavone), its name derived from the Latin term "Quercetum," meaning Oak Forest. It belongs to category flavonol, quercetin is a dietary compound that cannot be synthesized by the human body.

Physically, quercetin is a yellow-colored compound with limited solubility in hot water, moderate solubility in alcohol and lipids, and insolubility in cold water. Notably, quercetin is one of the most extensively utilized bioflavonoids for addressing metabolic and inflammatory disorders. Rutin (3,3',4',5,7-pentahydroxyflavone-3-rutinoside), also known as vitamin P, is a naturally occurring glycoside of flavonoids, widely present in plant-based sources. Rutin exhibits a broad range of biological and pharmacological activities, including antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial properties.

II. Materials and Methods

A double beam UV-spectrophotometer (LABINDIA Analytical UV3200), attached to a computer system. Digital balance , volumertric flask, micropipettes, beakers were used in the experiment. The absorption spectra for both the drugs were performed using quartz cell of one centimeter in the range of 200-400nm of wavelengths. Both the samples were accurately weighed with the help of digital balance machine.

Reagents and chemicals

Rutin was purchased from Central Drug House (P) Ltd, Quercetin purchased from Yucca Enterprises, Mumbai. All other reagent and chemicals procured are of standard grade from sigma Aldrich Pvt.Ltd. Ethanol was used to dissolve the drug as both drugs are lipophilic in nature

Method development

Selection of solvent

Rutin and quercetin were soluble in ethanol and phosphate buffer 7.4. before performing the experiment solubility of both drugs are evaluated as per standard criteria. Ethanol, which is available and cheap. The solubility of both drugs is good in ethanol. All the dilutions are prepared with phosphate buffer 7.4 which provide good results.

Selection of wavelength

Standard solutions of rutin and quercetin were scanned independently using spectroscopy across the 200-400 nanometer range to determine their absorption spectra. The absorption spectra of rutin and quercetin were overlaid to compare and gather data. Rutin and quercetin data were shown at maximum wavelength of 264nm and 371nm, respectively, using the simultaneous estimation approach.

Procedures

Preparation of standard stock solution and calibration curve

Preparation of standard stock solution:

According to Indian Pharmacopoeia standard stock solution for API were prepared for that 10 mg of Quercetin was dissolve in 100 ml of PBS 7.4 (100 µg/mL). Out of this stock 0.2-1.2 ml was pipetted and diluted up to 10 ml by solvent PBS 7.4 (2-12 µg/mL) and examined between 200-800 nm and 10 mg of rutin was dissolve in 100 ml of PBS 7.4 (100 µg/mL). Out of this stock 0.2-1.2 ml was pipetted and diluted up to 10 ml by PBS 7.4 (10-60µg/mL) and examined between 200-800 nm. The maximum absorbance was determined using UV-Vis Spectrophotometer (UV- 1700, Shimadzu, Japan) to confirm the λ max of the drugs.

Calibration curve

The standard stock solution prepared by dissolving 10 mg rutin in 10 ml ethanol. From the standard stock solution different dilutions were made ranging from 0-12ug/ml using PBS. Then absorbance is taken by UV spectrophotometer (LABINDIA Analytical UV3200) at 264nm. The UV calibration curve for RUTIN in PBS at

a concentration range 0-12µg/ml was found to be linear. Similar standard stock solution prepared for quercetin and it was found that UV calibration curve in PBS at a concentration 0-12 µg/ml is linear.

Simultaneous Equation method (SE)
From the standard stock solutions both drug (100 µg/mL), were taken and made it to final concentration of 2-12 µg/ml. Absorbance was measured at both the wavelengths by using solvent as blank. The reading was taken in triplicate. Absorbance maxima of both the drugs were recorded at both the wavelengths. The concentration was determined by using simultaneous equation method.

Simultaneous equation method formula
By using the below equations the concentrations in the samples were obtained
CX= A1ay2 – A2ay1 / ax1ay2 – ax2ay1 Eq. 1
CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1 Eq. 2
where A1 and A2 are absorbances of mixture at 253 nm and 249nm respectively, ax1 and ax2 are absorptivities of Quercetin at λ1 and λ2 respectively, ay1 and ay2 are absorptivities of rutin at λ1 and λ2 respectively, Cx and Cy are concentrations of Quercetin and Beta-sitosterol respectively.
A1 = ax1Cy + ay1Cx (At 371 nm)
A2 = ax2Cx + ay2Cy (At 264 nm)
A1 = absorbance value of the sample solution at 371 nm
A2 = absorbance value of the sample solution at 264 nm
ax1 = absorptivity of Quercetin at 371 nm
ax 2 = absorptivity of Quercetin at 264 nm
ay1 = absorptivity of Rutin at 264 nm
ay2 = absorptivity of Rutin at 371 nm
Cy = concentration of the rutin in µg/ml
Cx = concentration of the Quercetin in µg/ml

Determination of Iso-bestic point and selection of suitable Wavelength
An Iso-bestic λ point (a wavelength of equal absorptivity of the two components) was determined by taking overlain spectrum of the solutions Quercetin and Rutin of different concentrations in PBS 7.4 at UV range against the solvent blank. From the overlain spectra of the two drugs, it was found that Quercetin showed λ max at 371 nm and Rutin showed λ max at 264 nm.

The individual concentration range for beer-lambert was found 2-12 µg/ml for both Quercetin and Rutin at 371 nm and 264 nm with correlation coefficient 0.999 and 0.993. UV scan of 2-12µg/ml solution of Quercetin and Rutin combination showed the absorption maxima at 371 nm, 264 nm and 384 nm. The simultaneous estimation was done to check the interference between both the drugs at the λ max of one another.
By substituting absorbance and absorptivity values in simultaneous equation, Cx and Cy were calculated, Cx: 2.82 µg/ml, Cy: 4.57 µg/ml. The Linearity was observed by the linear regression equation method for Quercetin and Rutin in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. It was validated as per ICH guidelinesICHQ2 (R1).

Preparing the sample Solution
Topical gel formulation (1ml) containing equivalent to 5 mg of both drugs was transferred to volumetric flask and then drug dissolved in ethanol. The test solution was then filtered with the help of filter paper. Then solution diluted to get appropriate concentration of 30 µg/ml of rutin and quercetin, each the absorbance of test solution were determine at 264nm and 371nm against blank.

VALIDATION OF SPECTROSCOPIC METHOD
1. **Linearity:** For each drug sample, Serial dilutions of standard stock solutions were analyzed as per the procedure. The Beer Lambert's law was found to be in concentration range of 0-12 µg/ml for rutin and quercetin respectively. The linearity data for the method are summarized in Table
2. **Accuracy:** To determine the accuracy of the selected method, recovery studies were conducted at 80, 100 and 120% of the test concentration, in accordance with ICH guidelines. Each recovery level was analysed in triplicate.. The results of the recovery studies are summarize in **Table**

3. **Precision:**
   Interday and intraday precision: The Interday and intraday precision were evaluated by analyzing 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.**

4. **Limit of detection:** The detection limit was determined by analyzing samples with known concentrations of the analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$LOD = 3.3\sigma / S$$

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

5. **Limit of quantitation:** The quantitation limit is generally evaluated by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected with acceptable Precision and accuracy.

$$LOQ = 10\sigma / S$$

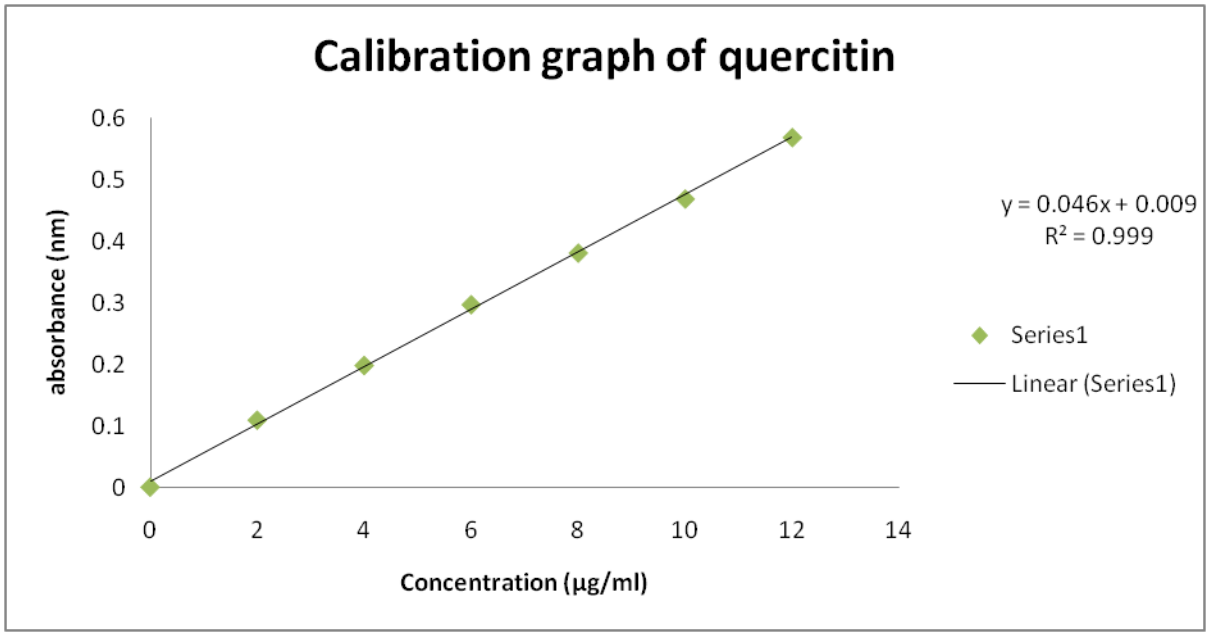
   Where σ =the standard deviation of the response S = the slope of the calibration curve

III. RESULTS AND DISCUSSION

The linearity ranges for rutin and quercetin were established as 0-12 µg/mL and 0-12 µg/mL, respectively, at their respective selected wavelengths. The calibration curves exhibited excellent correlation coefficients of 0.999 for rutin at 264 nm and 0.99 for quercetin at 371 nm. The regression analysis revealed good linearity at the respective wavelengths, indicating that the proposed methods can accurately detect small changes in drug concentration. The results of the recovery study further validated the accuracy and reliability of the proposed methods

Calibration curve

Graph 1: Calibration curve for quercetin at pH 7.4



Graph2: Calibration curve for Rutin

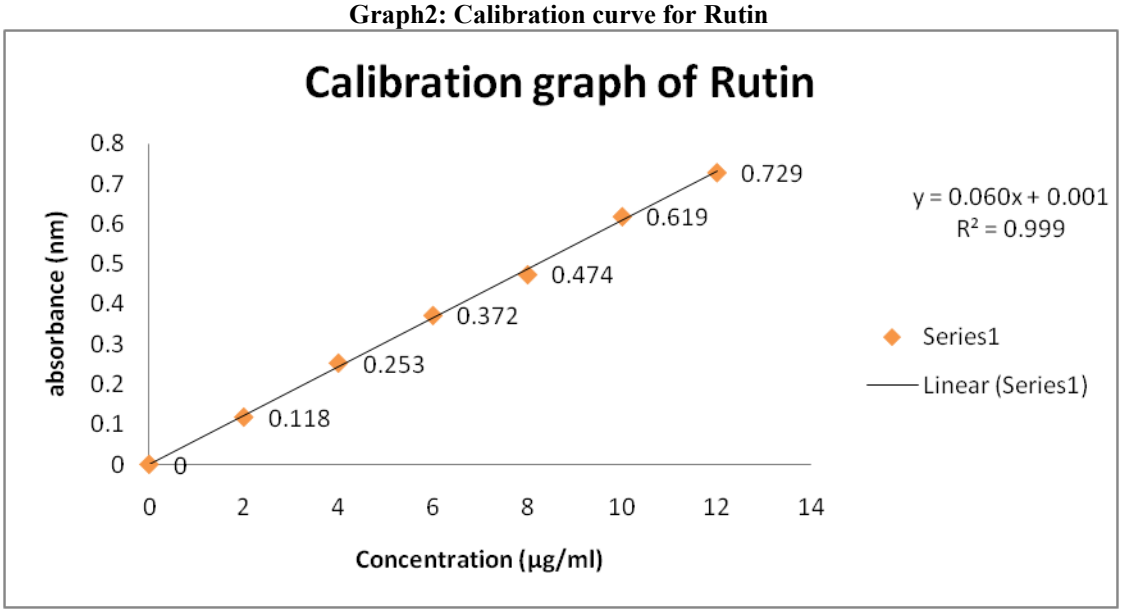
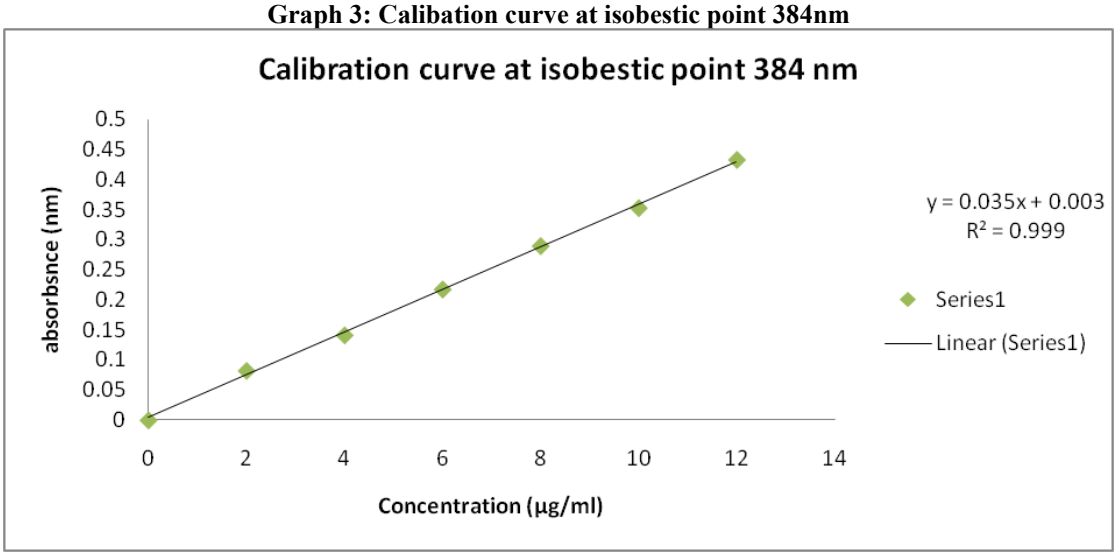


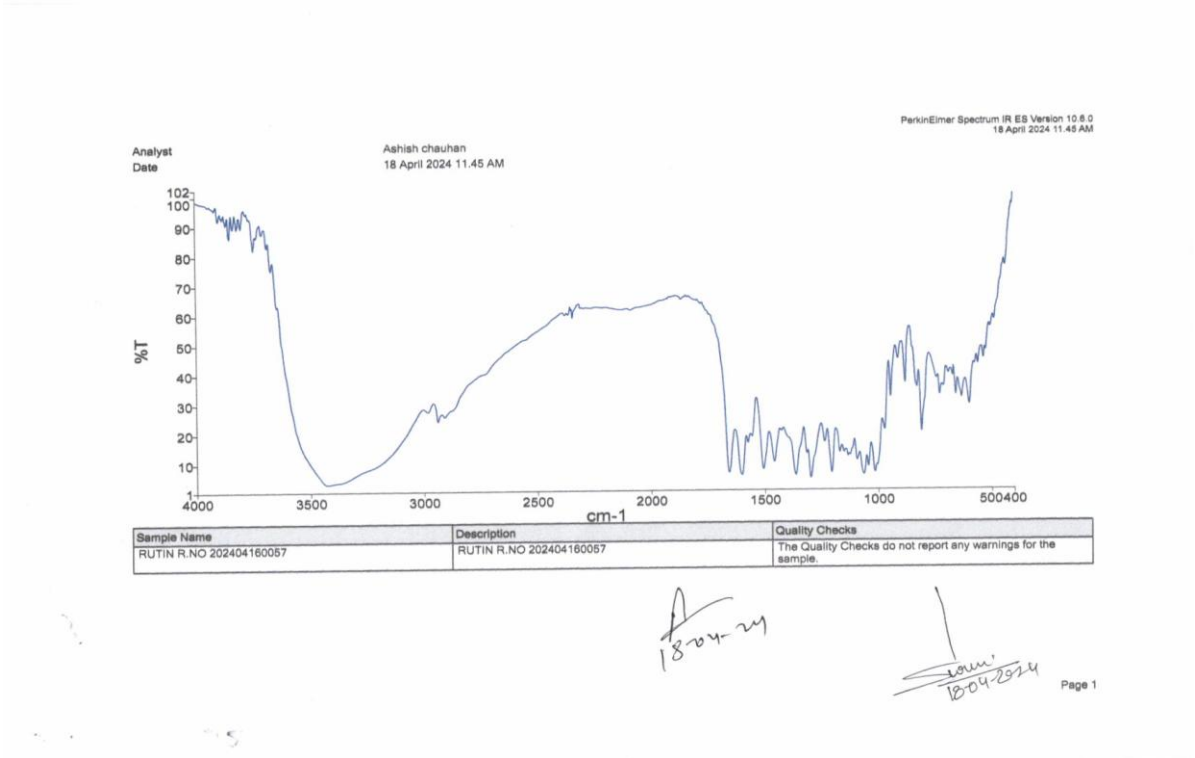
Table 1: showing the absorbance at Isobestic point

Concentration (µg/ml)	Absorbance (384 nm)
2	0.061
4	0.141
6	0.227
8	0.289
10	0.352
12	0.465
Mean	0.255
SD	0.145
%RSD	56.9

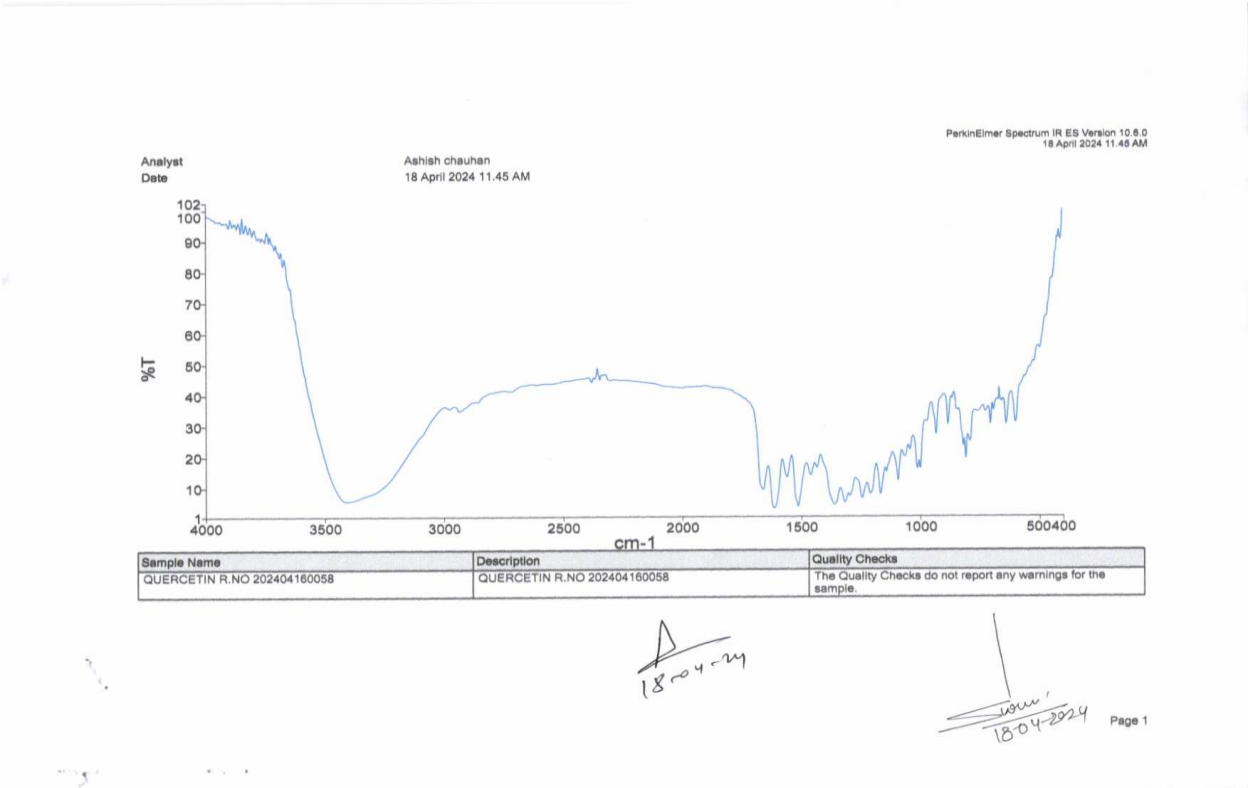
Graph 3: Calibation curve at isobestic point 384nm



RUTIN FTIR SPECTRUM



QUERCETIN FTIR SPECTRUM



Graph 4: Overlay spectra of Quercetin and Rutin with Iso absorptive point

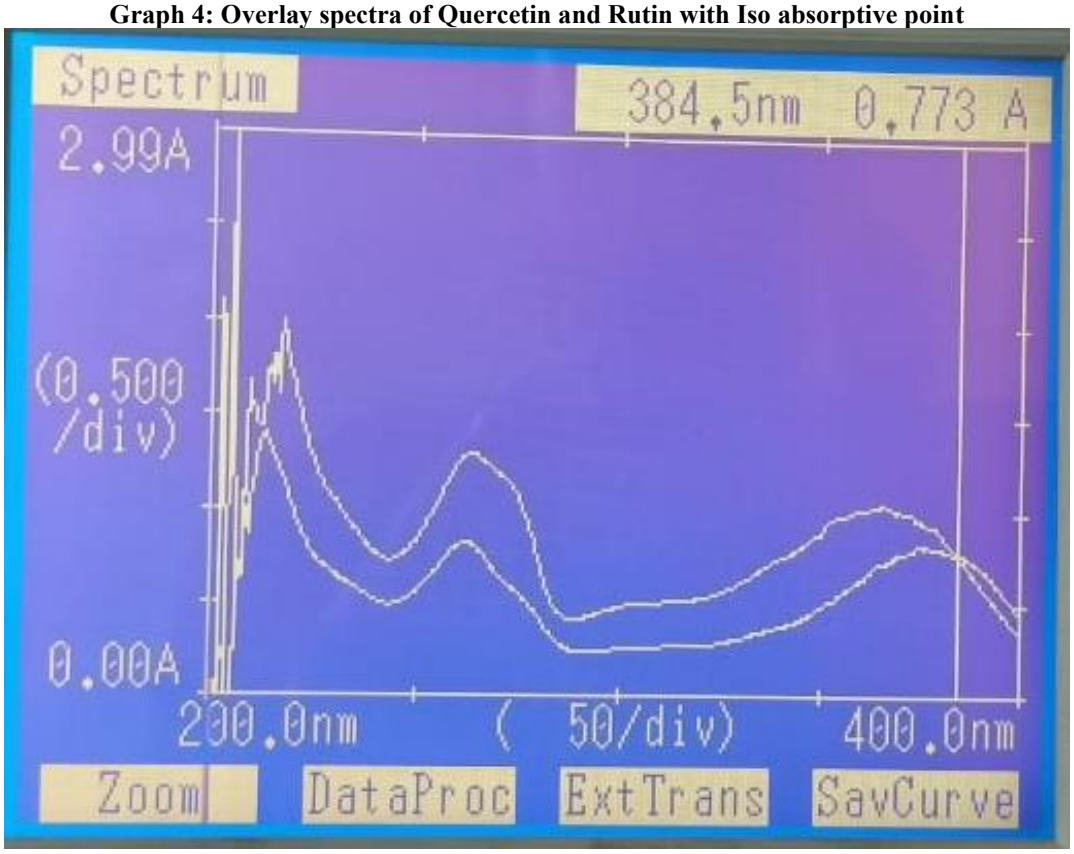


Table 2: Regression Characteristics

Parameters	Rutin	Quercetin
Wavelength	264nm	371nm
Linearity Range	0-12µg/ml	0-12µg/ml
Regression Equation (y = mx + c)	Y=0.060x+0.001	Y=0.046x+0.009
Slope (m)	0.060	0.046
Intercept (c)	0.001	
Correlation Coefficient (R^2)	0.999	0.997

Table 3: Data For Linearity

S. No	Linearity range of Rutin		Linearity range of Quercetin	
	Conc. in (µg/ml)	Absorb. at 264nm	Conc. in (µg/ml)	Absorb. at 371nm
1	0	0 ± 0	0	0 ± 0
2	2	0.118± 0.15	2	0.129±0.014
3	4	0.253±0.001	4	0.221±0.015
4	6	0.372 ±0.0015	6	0.311±0.021
5	8	0.474±0.0025	8	0.401±0.014
6	10	0.619±0.026	10	0.469±0.016
7	12	0.721±0.040	12	0.562±0.013

Table 4: Results of all parameters

S.NO	Parameters	Simultaneous Estimation Method	
		Rutin	Quercetin
1	Linearity Range	0-12µg/ml	0-12µg/ml
2	Correlation Coefficient (R^2)	0.999	0.997
3	Precision	%RSD	%RSD

4	**Intraday Precision**	0.0163	0.014
5	**Interday Precision**	0.0080	0.0048
6	**Repeatability**	1.67	1.76
7	**Accuracy**	**%Recovery**	**%Recovery**
8	**80%**	99.07±0.005	103±0.03
9	**100%**	103±0.059	103±0.073
10	**150%**	102±0.044	101 ±0.530
11	**Limit of detection (**µg/ml)	0.0264	0.1525
12	**Limit of quantification (**µg/ml)	0.0922	0.4248

Table 5: Results of Interday and Intraday Precision

	Interday Precision		**Intraday Precision**	
Drug	**% Amount found± SD**	**%RSD**	**%Amount found±SD**	**%RSD**
Rutin	96.14±0.0086	0.0080	94.45±0.0172	0.0163
Quercetin	103.68±0.0069	0.0048	100±0.013	0.014

Table 6: Results of Accuracy study

Concentration of the drug added to the formulation	**Rutin**		**Quercetin**	
	Recovery±SD	**%RSD**	**Recovery±SD**	**%RSD**
80%	99.07±0.005	0.114	103±0.03	0.040
100%	103±0.059	0.114	103±0.073	0.133
120%	102±0.044	0.106	101 ±0.530	0.396

IV. Conclusion

The simultaneous method for the estimation of both drugs is a crucial aspect of pharmaceutical analysis, ensuring the quality and efficacy of pharmaceutical product. A simple, accurate and precise method was validated for the simultaneous estimation of drugs. This study provides a reliable and efficient method for the simultaneous estimation of drugs. The developed method was successfully performed and validated.

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