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Method Development and Validation of UV Spectrometry for Quercetin Extracted From Dried Onion Peel

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Abstract: *The purpose of this research is to create a simple, accurate, precise, and affordable UV spectroscopic method for determining Quercetin. For the simultaneous determination of Quercetin, a modern UV spectrophotometric approach that is quick, easy to use, and accurate has been established. According to ICH Q2B criteria, many analytical performance parameters, including linearity, precision, accuracy, specificity, the limit of detection (LOD), and the limit of quantification (LOQ), were determined. The method is reliable and selective for the estimation of Quercetin, according to statistical analysis of the data. The developed method was effectively used for the determination of Quercetin in herbal plants and in commercial formulations.*

Keywords: UV Spectroscopy, Quercetin, ICH, LOD, LOQ

I. INTRODUCTION

A number of issues emerge during the analysis of quercetin. The analytical challenges related to quercetin analysis begin with its solvent solubility behavior when exposed to particular solvents thus causing difficulties in creating proper sample concentrations. The instrument detects the compound with imprecise sensitivity or encounters calibration problems to generate incorrect compound measurements. The analytical compound known as quercetin shows susceptibility to degradation when exposed to heat or light which leads to analytic loss. Different extraction protocols result in untraceable outcomes which affect the device data between various sample collection batches.

Advanced methods for quercetin analysis through IR (Infrared Spectroscopy), NMR (Nuclear Magnetic Resonance), Mass Spectrometry (MS) and HPLC (High-Performance Liquid Chromatography) prove to be both technically demanding and expensive to perform. Highly qualified employees are necessary for running these complex analytical methods.

The developed analysis method enables assessment of quercetin extracted from onion peel with both simple operation and exact and accurate findings. The protocol offers cost-effective operations and user-friendly knowledge for quercetin analysis in commercial onion peel samples.

Instrumentation:

Fig. No. 1 UV Spectrophotometer (Shimadzu UV -1780, Japan)

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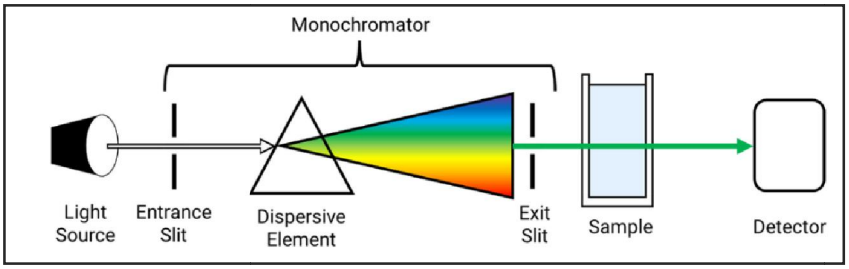

Fig. No. 2 UV Spectrophotometer Instrumentation

For this research the scientists utilized a double beam UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan). It included spectra manager software UV Probe through the computer system. This instrument combines a 20W halogen lamp and a deuterium lamp which enable measurement between wavelengths 190 to 1100 nm. This instrument features a desktop design that is easy to shift locations and comes with a bright LCD screen that folds open for use. The device operates under the requirements defined by US, European, and Japanese Pharmacopoeia standards.[1]

The method of absorption spectroscopy known as UV spectroscopy detects absorption of ultra-violet light in the wavelength region between 200 and 400 nm. by the studied molecule. The absorption of ultra-violet radiations leads to electron excitations from the ground state to higher energy states. Radiations that fall within the ultra-violet range produce energy identical to the separation in energy between levels of molecular states including ground state.[2]

The spectral measurements used quartz cuvettes that measured 1 cm for all measurements. The acquisition of spectra took place using these instrumental specifications: Wavelength range from 200 to 400 run. Researchers obtained all weights through the use of (Adventurer™ Ohaus) electronic balance devices. The validation study made use of calibrated volumetric glassware manufactured by Borosil®.[3]

Quercetin is a plant flavonol compound which occurs throughout various kinds of fruits and vegetables and leaves. Plants produce the plant flavonol Quercetin which exists in various fruits and vegetables as well as leaves and grains. It acts as antioxidant. The substance functions as a broad-spectrum protein kinase enzyme blocking agent. Scientists documented estrogenic properties which stem from the receptor activation characteristics of quercetin. This compound serves physicians in treating multiple medical conditions including heart diseases while benefiting patients who suffer exercise-induced respiratory problems as well as those with high cholesterol levels diabetes asthma and gout and patients facing cancers of lung ovarian and pancreatic origin. The international name designated by IUPAC for Quercetin consists of 2- (3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy4H-chromen-4- one (Fig. 2). The substance exists as yellow crystalline powder with C15H10O7 as its molecular formula and 302.236 g/mol as its molecular weight. The pure substance Quercetin displays a melting point at 316 °C. The compound exhibits high solubility in ether and methanol together with solubility in ethanol, acetone, pyridine, and acetic acid. We have established a straightforward UV and HPLC analysis technique which can measure Quercetin content in bulk drug and microspheres in the current investigation.[4,5]

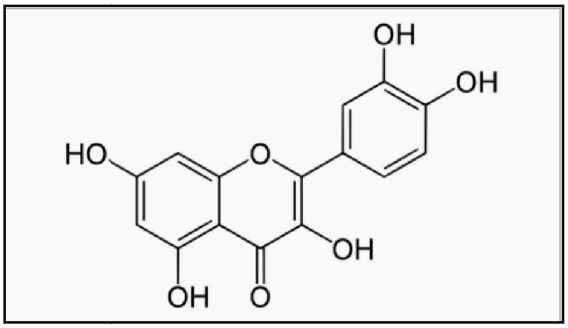

Fig. No. 3 Chemical Structure of Quercetin

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II. RATIONALE

Purpose of the Research

The objective is to develop an affordable and accurate method through UV spectroscopy for measuring Quercetin extracted from onion peel waste. An analytical system needs development which provides quick operation together with simple maintenance and accurate detection of Quercetin samples in one measurement cycle.

Significance of Quercetin

- Multifunctional Plant Flavanol with diverse health benefits
- Acts as an antioxidant
- Non-specific protein kinase enzyme inhibitor
- Potential estrogenic activities

Medical Applications

The testing approach provides therapy for different medical situations including:

o Heart diseases
o Exercise-induced respiratory problems
o High cholesterol
o Diabetes
o Asthma
o Gout

Methodological Importance

The method fulfills the requirements of ICH Q2B criteria- Q2B Validation of Analytical Procedures: Methodology.
The assessment included multiple testing performance criteria for evaluation.

• Linearity
• Precision
• Accuracy
• Specificity
• Limit of Detection (LOD)
• Limit of Quantification (LOQ)

Broader Impact

The technique represents a dependable research approach to analyze Quercetin content in onion peel extracts for academic researchers as well as industrial laboratories.

OBJECTIVES

-A UV spectrometry system needs development for detecting Quercetin compounds in onion peels extracts.
-The method requires validation to confirm the accuracy reliability as well as reproducibility of the UV spectrometric method when used for Quercetin onion peel analysis through UV spectroscopy.

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Drug Profile- Quercetin

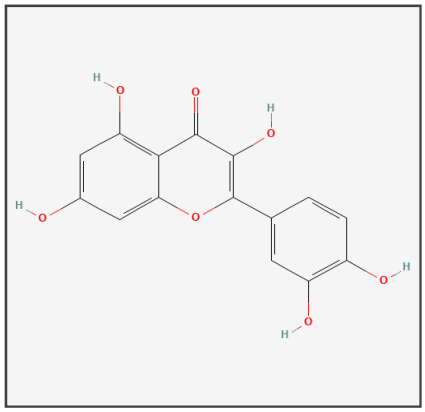

Table 1: Drug profile

Chemical Name	**2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one**
Molecular Formula	$C_{15}H_{10}O_7$
Molecular Weight	302.23 g/mol
Description	appears as yellow needles or yellow powder
Solubility	Very soluble in ether, methanol; soluble in ethanol, acetone, pyridine, acetic acid
Melting Point	316.5 °C
pKa value	7.17
Log P	1.5
Storage	Protect this chemical from exposure to light. Keep the container tightly closed under an inert atmosphere, and store under refrigerated temperatures

III. MATERIALS AND RESEARCH METHODOLOGY

Row Plant Material

The researcher obtained dried onion skin samples (Allium cepa) in mid-January until the end of February from Kagal which falls within Kolhapur District in Maharashtra State of India.

Table No.-2 Apparatus used for method development:

Sr. No.	**Material**	**Particular**
1.	Beaker	J-SIL, India
2.	Conical Flask	PioneerImpex, Ahemdabad
3.	Test Tube	J-SIL, India
4.	Measuring Cylinder	H.L.Scientific industry 744
5.	Stirrer	J-SIL, India
6	Reflux Condenser	J-SIL, India

Table No.-3 Instruments used for method development:

Sr. No.	**Name of Instrument**	**Manufactures**
1.	V-1800 UV-visible spectrophotometer (software UV probe 2.70 version)	Shimadzu Corporation UV-visible double beam spectrophotometer (Shimadzu 1800)
2.	Digital Balance	Digital electronic balance citizen & Contact (CY220 &CY223)
3.	V-1700 UV-visible spectrophotometer (software UV probe 2.33 version)	Shimadzu Corporation UV-visible double beam spectrophotometer (Shimadzu 1700)

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Table No.-4 chemicals used for method development:

Sr. No.	Material	Quantity
1.	Ethyl Acetate	500 ml
2.	Methanol	150 ml
3.	Distilled water	1000 ml

All the glassware's which was used were made up of Borosilicate and they were calibrated.

IV. RESEARCH METHODOLOGY

Preparation of crude extracts

1. **Preparation:**

Collect approximately 50 grams of dry, brown onion skins.

Cutting the onion skin pieces into smaller fragments will make extraction more efficient.

2. **Solvent Selection:**

The research uses 500ml of ethyl acetate solvent because this substance successfully reduces unwanted contaminants during extraction.

The onion skin should go into a flask followed by adding solvent while heating the mixture through the reflux process. A condenser should be installed to avoid solvent escape.

3. **Extraction Process**:

Allow the mixture to sit under reflux conditions during a 2-hour period to dissolve the quercetin throughout the solution. The solution develops a yellow color due to the extraction of quercetin.

4. **Separation:**

When the solution reaches completion transfer it to a 2-neck round bottom flask that will serve for distillation.

A heating mantle will help evaporate the solvent until only non-volatile compounds including quercetin remain in the flask.

5. **Purification:**

The black tar-like insoluble material needs to be removed through solution filtration.

After filtering the solution through a watch glass the yellow-brown powder will remain as an end product.

6. Recrystallization:

Recrystallization with Ethanol Solution is done to get pure drug powder.

Identification test for quercetin

1	Test	Observation	Inference
1	Ferric Chloride Test Ethanolic extract + Add 2–3 drops of neutral ferric chloride solution.	green or blackish-green color observed	phenolic hydroxyl groups presence in quercetin
2	Shinoda Test (Magnesium + HCl) 2 mL ethanolic solution of quercetin, add a small amount of magnesium ribbon followed by a few drops of concentrated HCl.	Pink to red coloration observed	confirms flavonoid structure like quercetin
3	Lead Acetate Test Dissolve 10 mg quercetin in 2 mL ethanol. Add 1 mL of 10% lead acetate solution.	Yellow precipitate observed	flavonoids like quercetin are present

Table No.- 05 Identification test for quercetin

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METHOD DEVELOPMENT: UV SPECTROPHOTOMETRIC METHOD

From the above stock solution, 2 ml of the drug solution is transferred into a 10 ml volumetric flask, and the volume is made up to the mark with the methanol and water mixture to achieve a concentration of 20 µg/ml. The sample is scanned with a UV-visible spectrometer in the range of 200-400 nm against the methanol and water mixture as a blank. The wavelength corresponding to the maximum absorbance is noted, which is its λ_{max}.

Validation of Developed Method:

Determination of λ_{max} for Quercetin: From the stock solution, 2 ml of the drug solution is transferred into a 10 ml volumetric flask, and the volume is made up to the mark with mixture of methanol and water to prepare a concentration of 20µg/ml. The sample is scanned using UV-visible spectrometer in the range of 200nm-400 nm against the methanol and water mixture as a blank mixture. The wavelength corresponding to the maximum absorbance is noted, which is its λ_{max}.

Linearity: Linearity is performed by using the stock solution at different concentrations with 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml, 2 ml which results in concentrations of 4µg, 8µg, 12µg, 16µg, and 20 µg, respectively & graph is plotted.

Accuracy: To check the accuracy of the proposed method, recovery studies are carried out using 0.5 ml, 1 ml, and 1.5 ml to achieve 50%, 100%, and 150% recoveries, respectively.

$$\textbf{\% recovery} = \frac{\textbf{concentration found}}{\textbf{concentration used}} \times \mathbf{100}$$

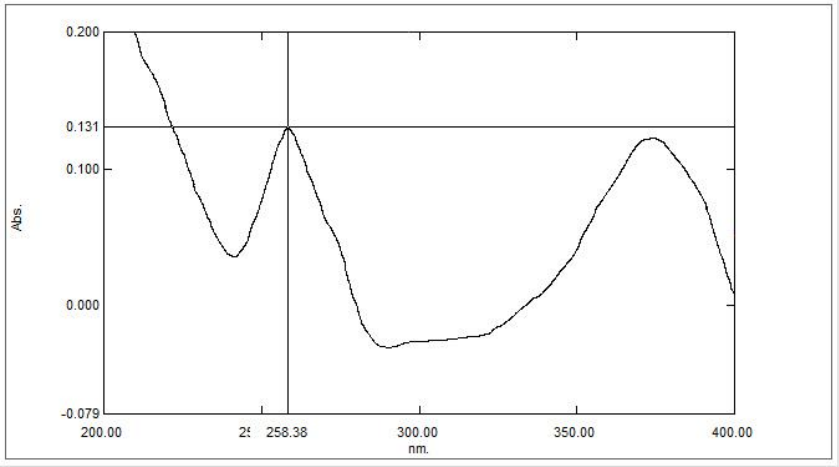
Precision: The interday and intraday precision are determined by assaying the sample solution on the same day and at different time intervals, respectively.

Ruggedness : The ruggedness of the method is assessed by spiking the standard three times with different analysts using the same instrument.

Robustness: The ruggedness of the method is assessed by spiking the standard three times with different analysts using the same instrument.

V. RESULT AND DISCUSSION:

Determination of λ_{max} for Quercetin: The UV-Visible spectrum of Quercetin in Methanol and water showed a λmax at **258.38nm**, which is in agreement with literature values (typically 258 to 380 nm). First peak at 258.38nm and 2nd peak 370nm.



Graph No.-1 UV absorbance **Vs** wavelength **Graph**

Linearity: By determining absorbance of sample prepared concentration, Linearity data was found is tabulated in table no.-6 and R2 value was found by plotting graph is - 0.9984.

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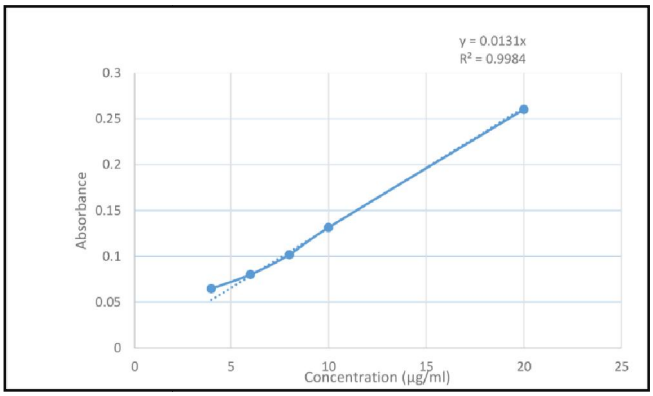
Table No. 6 Linearity data

Sr. No	Concentration µg/ml	Absorbance at 258nm
01	0.4ml (4µg/ml)	0.0647
02	0.6ml (6µg/ml)	0.0801
03	0.8ml (8µg/ml)	0.1018
04	1ml (10 µg/ml)	0.1314
05	2ml (20 µg/ml	0.2602



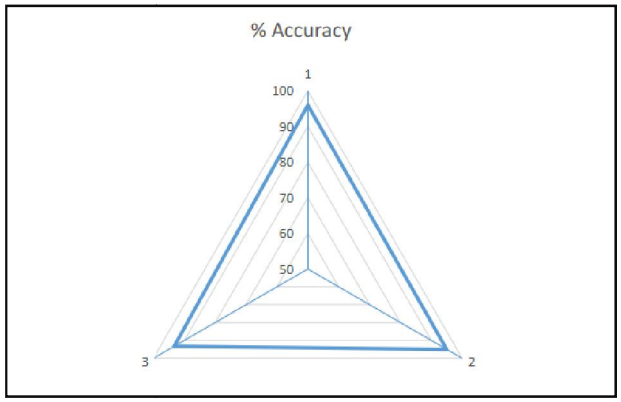
Graph No.-2 UV absorbance Vs concentration Graph for linearity

Accuracy: To check the accuracy of the proposed method, recovery studies are carried out using 0.5 ml, 1 ml, and 1.5 ml to achieve 50%, 100%, and 150% recoveries, respectively. All readings are tabulated in Table No. 07

$$\textbf{\% recovery} = \frac{\textbf{concentration found}}{\textbf{concentration used}} \times \mathbf{100}$$

Table No. 7 Accuracy data

Sr. No	Sample ID with concentration used	Concentration found at 258nm	% Accuracy
01	50 % (5 µg/ml)	4.8 µg/ml	96 %
02	100%(10 µg/ml)	9.5 µg/ml	95 %
03	150%(15µg/ml)	14 µg/ml	93.33 %



Graph No. 3 % Accuracy

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Precision: The interday and intraday precision are determined by assaying the sample solution on the same day and at different time intervals, respectively. All readings are mentioned in Table No. 08.

Table No.08 Precision data

Sr. No	Sample ID	Absorbance
01	Morning	0.1192
02	Afternoon	0.1196
03	Evening	0.1198

Ruggedness: The ruggedness of the method is assessed by spiking the standard three times with different analysts using the same instrument. All readings are mentioned in Table No. 09.

Table No.09 Ruggedness data

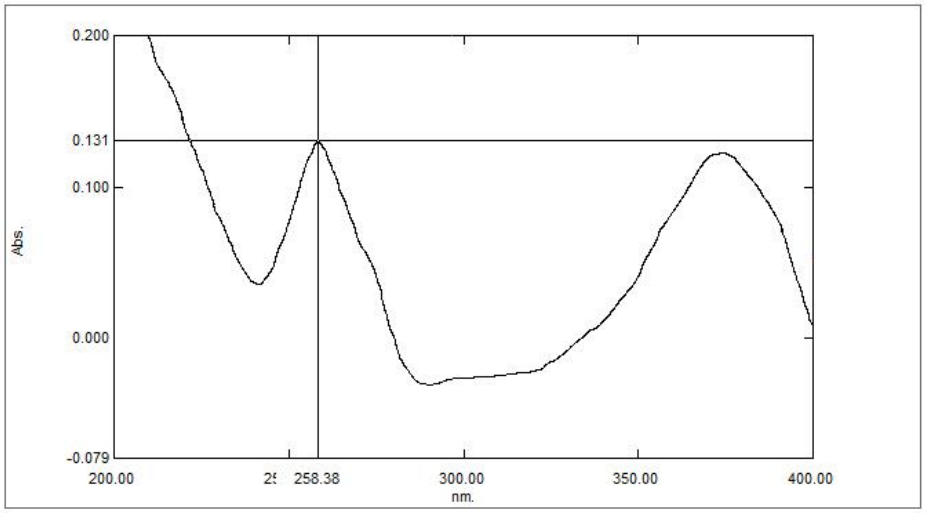
Analyst	Wavelength	Absorbance
Analyst 01	258nm	0.1340
Analyst 02	258nm	0.1314
Analyst 01	258nm	0.1290

Robustness: The robustness of the method is assessed by spiking the standard two times with different instruments. All readings are mentioned in Table No. 10.

Table No. 10 Robustness data

Instruments	Wavelength	Absorbance
Instrument 01	258	0.1314
Instrument 02	260	0.1290

Instrument 01



Graph No.-4 UV absorbance Vs wavelength Graph

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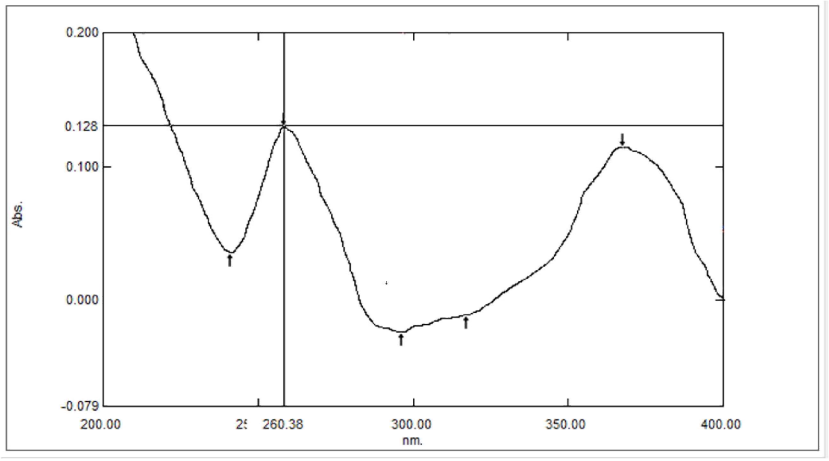
Instrument 02



Graph No.-5 UV absorbance Vs wavelength Graph

VI. CONCLUSION

This research develops a basic and durable UV spectrophotometric analysis procedure for quercetin extracted from onion peels through UV spectroscopy. A main benefit of this technique includes low expense together with its straightforward application of simple solvents and well-established end results. The analytical method meets all requirements of the ICH guidelines for validation standards. The examined data proves that this analytical approach demonstrates high precision together with reliability

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