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CHARACTERIZATION AND QUANTIFICATION OF ACTIVE COMPOUNDS CAFFEINE AND QUERCETIN BY UV–VISIBLE SPECTROSCOPY

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ABSTRACT

The present study focuses on the characterization and quantification of the active compounds caffeine and quercetin using a simple and reliable UV–Visible spectrophotometric method. Both compounds were initially characterized through organoleptic properties, solubility studies, melting point determination, pH analysis, and FTIR spectroscopy, confirming their identity and purity. Caffeine was observed as a white, odorless crystalline powder, while quercetin appeared as a yellow crystalline solid, with both showing physicochemical properties consistent with standard references. UV–Visible spectrophotometric analysis revealed distinct maximum absorbance (λmax) values of 273 nm for caffeine and 373 nm for quercetin, with an isobestic point at 263 nm, indicating suitability for simultaneous analysis. The methods demonstrated good linearity within Beer's law ranges of 5–25 µg/ml for caffeine and 10–50µg/ml for quercetin, with correlation coefficients of 0.9912 and 0.9945, respectively. The slope values (0.0636 for caffeine and 0.0643 for quercetin) indicated good sensitivity of the method. Validation parameters confirmed the reliability of the method, with low %RSD values for precision studies, including repeatability (0.550% for caffeine and 1.398% for quercetin), intraday (0.642% and 0.937%), and interday (0.549% and 0.820%) variations. Ruggedness and robustness studies showed %RSD values below 2%, indicating consistency under different analysts and temperature conditions. The method also exhibited good sensitivity, with LOD values of 1.220µg/ml (caffeine) and 2.113µg/ml (quercetin), and LOQ values of 3.698 µg/ml and 6.404µg/ml, respectively. In conclusion, the developed UV–Visible spectrophotometric method is simple, accurate, precise, and cost-effective for the characterization and quantitative estimation of caffeine and quercetin. The method complies with ICH validation guidelines and is suitable for routine analysis in pharmaceutical and quality control laboratories.

KEYWORDS: UV Spectrophotometry; Percent Recovery; Method Validation; ICH Q2(R2); Method Deployment.

1. INTRODUCTION

The identification and quantitative estimation of bioactive compounds are essential steps in pharmaceutical analysis, quality control, herbal drug standardization, and formulation development. Among the various analytical techniques available, UV–Visible spectroscopy is one of the most widely employed instrumental methods because of its simplicity, accuracy, rapidity, cost-effectiveness, and sensitivity **(Kumari *et al.*, 2016).** It is extensively used for the characterization and quantification of compounds that possess chromophoric groups capable of absorbing ultraviolet or visible radiation. In pharmaceutical and phytochemical analysis, UV–Visible spectroscopy plays a significant role in determining the concentration, purity, and stability of active constituents present in synthetic and natural products **(Arora *et al.*, 2023).**

UV–Visible Spectroscopy is an analytical technique based on the absorption of ultraviolet and visible light by molecules. When electromagnetic radiation in the UV or visible region passes through a sample, certain wavelengths are absorbed by the compound depending on its electronic structure. The absorption occurs because

electrons in the molecule are excited from lower energy states to higher energy states **(Alaboodi *et al.,* 2025).**

Caffeine is a naturally occurring alkaloid belonging to the methylxanthine class of compounds. Chemically, it is known as 1,3,7-trimethylxanthine and is commonly found in coffee beans, tea leaves, cocoa beans, kola nuts, and several energy beverages **(Emerson *et al.,* 2019).** Caffeine is one of the most widely consumed psychoactive substances in the world because of its stimulating effect on the central nervous system. It is extensively utilized in pharmaceutical formulations due to its ability to enhance alertness, reduce fatigue, and improve cognitive performance **(Nwokike *et al.,* 2021).**

Pharmacologically, caffeine acts primarily as an adenosine receptor antagonist, resulting in increased neuronal activity and stimulation of the brain. It is also used therapeutically in analgesic combinations, migraine treatments, respiratory stimulants, and neonatal apnea management. In addition to pharmaceutical applications, caffeine is commonly incorporated into dietary supplements, soft drinks, sports beverages, and cosmetic products **(Kandasamy *et al.,* 2016).**

Quercetin is a naturally occurring flavonoid widely distributed in fruits, vegetables, leaves, flowers, and medicinal plants. It belongs to the flavonol subclass of flavonoids and is chemically identified as 3,3′,4′,5,7-pentahydroxyflavone. Quercetin is abundantly present in onions, apples, berries, grapes, citrus fruits, tea, and various herbal medicines **(Vollmannová *et al.,* 2024).** Quercetin has attracted significant scientific attention because of its diverse pharmacological and therapeutic properties. It exhibits potent antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, antihypertensive, cardioprotective, hepatoprotective, and immunomodulatory activities. The antioxidant property of quercetin is primarily due to its ability to scavenge free radicals and chelate metal ions, thereby protecting biological systems from oxidative stress and cellular damage **(Yang *et al.,* 2020).**

In pharmaceutical and herbal research, the quantification of quercetin is important for the standardization of herbal extracts and polyherbal formulations. Since the concentration of quercetin may vary depending on plant source, environmental conditions, extraction methods, and processing techniques, reliable analytical methods are necessary for accurate estimation and quality assurance **(Mansour *et al.,* 2025).**

The present study aims to characterize and quantify caffeine and quercetin using UV–Visible spectrophotometry by determining their absorption maxima, preparing calibration curves, and validating the analytical method.

2. MATERIAL AND METHOD

2.1 Chemicals

DMSO, Ethyl acetate, and Methanol, were obtained from Rankem, a reputable supplier of analytical reagents. Finar provided the Acetone and Chloroform. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Bio Liqua Pvt. Ltd., and Actylis.

2.2 Organoleptic properties

These characteristics create identity and purity parameters, monitor stability, identify degradation, and function as crucial quality control parameters. In this study Organoleptic Properties of pure compounds evaluated are color, odor, physical appearance, and state **(Bergström *et al.,* 2003).**

2.2.1 Solubility Study

Solubility studies are an essential part of analytical method development, as they help in selecting suitable solvents for the preparation of stable and reliable drug solutions. In UV–Visible spectroscopic analysis, the choice of solvent significantly influences the absorbance characteristics, sensitivity, and accuracy of the method. Proper solubility ensures uniform sample preparation, accurate dilution, and good linearity over the required concentration range. Therefore, different solvents such as methanol, ethanol, water, dichloromethane, and DMSO were evaluated during the solubility study to identify the most suitable medium for analysis **(Vázquez-Blanco *et al.,*2018).**

2.2.2 Melting point determination

The melting point was determined using sealed glass capillary tubes and an electrically heated melting point apparatus. The sample-filled capillary tube was heated gradually, with the temperature increased at 1–2°C per minute near the expected melting range for accurate observation of the melting point **(Singh *et al.,* 2023).**

2.3 Identification of pure drug-Fourier Transform Infrared (FTIR)

FTIR spectroscopy was used to identify the characteristic functional groups and confirm the molecular structure of the drug based on its unique infrared absorption spectrum. For analysis, the sample was prepared by mixing finely powdered drug with dried potassium bromide (KBr) to form a pellet, which was then analyzed using a Perkin Spectrum BX FTIR spectrophotometer over the range of 400–4000 cm^{-1}. Background scanning was performed to eliminate interference from atmospheric moisture and carbon dioxide, and the obtained spectrum was processed using baseline correction for accurate peak identification **(Siddique *et al.,* 2024).**

2.4 Method Development by UV spectroscopy

The amount of light absorbed by the sample was measured using UV-visible spectrophotometric

techniques to determine the concentration of solutes in the solution.

2.4.1 Preparation of standard solution

An accurately weighed quantity of Caffeine and Quercetin (10 mg) was transferred into separate10mL volumetric flasks. The drugs were dissolved in a small amount of analytical-grade methanol, and the final volume was adjusted to the mark with the same solvent to produce a primary stock solution of 1000µg/ml. To prepare the sub-stock solution (100µg/ml) of both the drugs, a 1ml aliquot of the primary stock was pipette into a second 10 ml volumetric flask and diluted to volume with methanol. From this sub-stock, a series of five working standards of Caffeine and Quercetin with concentrations ranging from 5 to 25µg/ml were prepared through appropriate serial dilutions separately **(Verma *et al.*, 2018).**

2.4.2 Determination of wavelength of maximum absorbance (λ_{max})

To analyze an appropriate wavelength, drug dilution were scanned in UV-Vis Spectrophotometer within the range of 200-800nm. The wavelength at which the molecule showed maximum absorption was called the lambda max of a particular drug **(Rani *et al.*, 2024).**

2.5 Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

2.5.1 Linearity and Range

Linearity of the developed UV spectrophotometric method was evaluated by preparing standard solutions in the concentration range of 5–25µg/mL. The absorbance of each concentration was measured, and calibration curves of absorbance versus concentration were plotted. Linear least squares regression analysis was performed, and the correlation coefficient (R^2 value) was used to assess the linearity and proportional relationship between concentration and absorbance **(Islam *et al.*, 2020).**

2.5.2 Precision

Precision was evaluated to determine the repeatability and reliability of the analytical method and was expressed as percent relative standard deviation (%RSD). According to ICH guidelines, precision studies included repeatability, intermediate precision, and reproducibility. Repeatability was assessed by recording absorbance six times for the selected concentration. Intermediate precision was determined by measuring absorbance in triplicate during morning, afternoon, and evening on the same day, while reproducibility was evaluated by analyzing the selected concentration in triplicate over three consecutive days **(Goswami *et al.*, 2022).**

2.5.3 Robustness

Robustness is defined as the capacity of a method to remain unaffected by small but deliberate variations in method parameters. The robustness of a method could be evaluated via various method parameters which include the percentage of organic solvent, pH, ionic strength, or temperature, and determining the effect on the results of the method To determine robustness of the developed method the absorbance in triplicates of the selected concentration was recorded at 10ºC, 25ºC, and 45ºC **(Ravisankar *et al.*, 2015).**

2.5.4 Ruggedness

To determine ruggedness of the developed method the absorbance of the selected concentration was recorded by three analysts in triplicates **(Killeen *et al.*, 2014).**

2.5.5 Limit of Detection

The limit of detection is defined as the lowest concentration of the analyte in the sample that can be detected, though not necessarily quantitated. Limit tests specify whether an analyte is above or below a certain value. LOD may be calculated based on the standard deviation (S.D.) of the response and the slope (A) of the calibration curve at levels approaching the LOD according to the formula.

LOD = 3.3*(S.D/A)

2.5.6 Limit of Quantitation

The limit of quantitation (LOQ) is defined as the detection of the lowest amount of analyte present in the sample. It is the parameter that gives the actual concentration of an analyte in a sample which can be determined with acceptable precision and accuracy under the stated operating conditions of the method during analysis. The calculation of LOQ is based on the standard deviation (S.D) of the response and the slope (A) of the calibration curve **(Ben-Tal *et al.*, 2000).**

The formula to calculate is;

LOQ = 10*(S.D/A)

3. RESULTS AND DISCUSSION

3.1 Organoleptic Properties of Caffeine and Quercetin.

Table 1: Organoleptic Properties of Caffeine and Quercetin.

Organoleptic Properties	Caffeine	Quercetin
Color	White	Yellow
Odor	Odorless	Odourless
Physical appearance	Crystalline powder	Crystaline powder
State	Solid	Solid

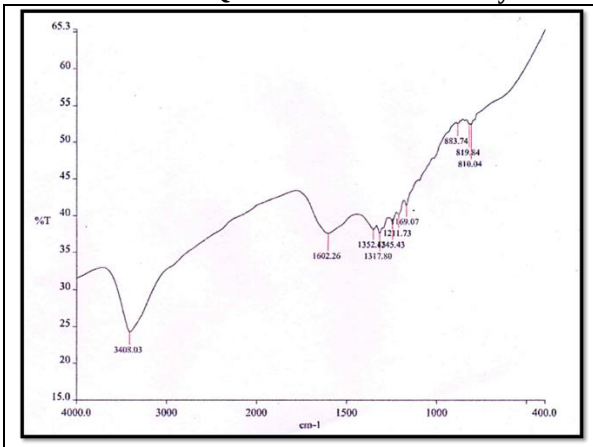
3.2 Melting point and pH of Caffeine and Quercetin

Table 2: Melting point and pH of Caffeine and Quercetin.

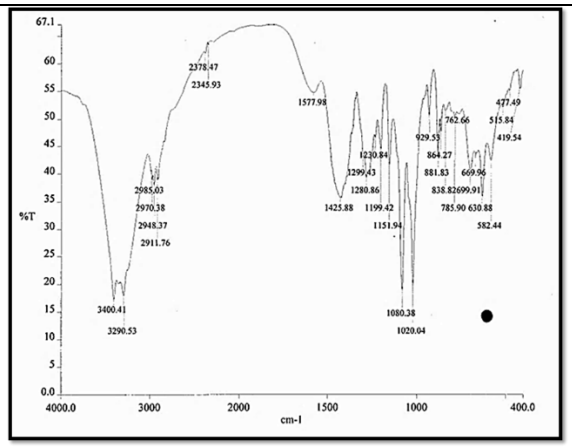
Drug	Reference range (Melting point)	Observation (Melting point)	Reference range (pH)	Observation (pH)
Caffeine	235–238 °C	237°C	5.5-6.9 pH	6.5pH
Quercetin	314-317°C	313°C	5-6 pH	5.4 pH

3.3 FTIR

3.3.1 Identification of Quercetin and Caffeine by FTIR.



Graph 1: Quercetin



Graph 2: Caffeine

Table 3: FTIR Interpretation of Quercetin.

Frequency Range	Group Absorption	Group	Compound Class
3500- 3400 (cm^{-1})	3408.03	N-H stretching	primary amine
1650-1600 (cm^{-1})	1602.26	C=Cstretching	conjugated alkene
1420-1330 (cm^{-1})	1352.42	O-H bending	alcohol
1390-1310 (cm^{-1})	1317.80	O-Hbending	phenol
1225-1200 (cm^{-1})	1211.73	C-O stretching	vinyl ether
1210-1163 (cm^{-1})	1169.07	C-Ostretching	ester

Table 4: FTIR Interpretation of Caffeine.

Frequency Range	Group Absorption	Group	Compound Class
3550-3200 (cm^{-1})	3400.41	O-H stretching	alcohol
3330-3250 (cm^{-1})	3290.53	N-H stretching	aliphatic primary amine
3000-2840(cm^{-1})	2985.03	C-H stretching	alkane
3000-2500 (cm^{-1})	2948.37	C-H stretching	alkyne
1650-1566 (cm^{-1})	1577.98	C=Cstretching	cyclic alkene
1440-1395 (cm^{-1})	1425.88	O-Hbending	carboxylic acid
1342-1266	1299.43	C-Nstretching	aromatic amine
1210-1163	1151.94	C-Ostretching	Ester

3.4 Recovery studies

Table 5: %recovery in spiked sample of Quercetin and Caffeine.

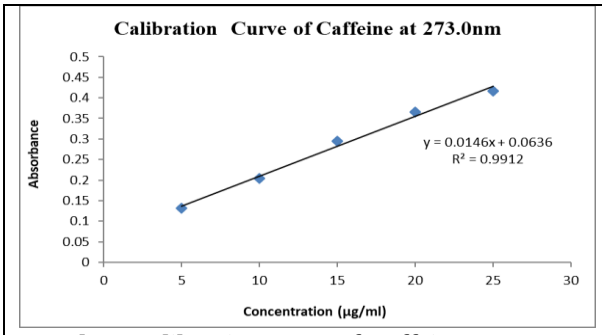
Pre-analyzed sample solution	Amount of drug added (n=3)	%Recovery	%RSD	Pre-analyzed sample solution	Amount of drug added (n=3)	%Recovery	%RSD
10	80%	99.55	1.46	**30**	80%	100.05	2.18
10	100%	100.28	1.51	**30**	100%	101.59	2.19
10	120%	99.40	1.59	**30**	120%	101.18	1.95

3.5 Method Validation

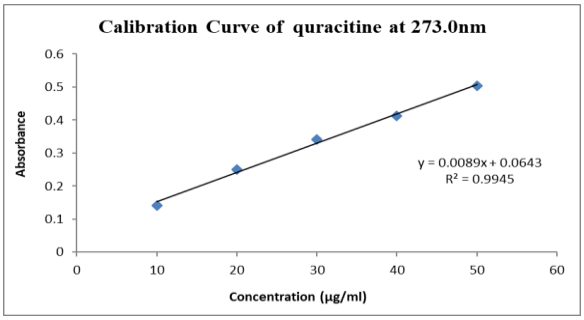
3.5.1 Linearity and range

Table 6: Calibration data of Caffeineat 273.0nm and Quercetin at 373.0nm.

Concentration (µg/ml)	Absorbance 1 at 273.0nm	Absorbance 2 at 273.0nm	Absorbance 3 at 273.0nm	Mean Absorbance 273.0nm	Absorbance 1 at 373.0nm	Absorbance 2 at 373.0nm	Absorbance 3 at 373.0nm	Mean Absorbance 373.0nm
	Caffeine at 273.0nm				Quercetin at 373.0nm			
5	0.112	0.132	0.152	0.132	0.141	0.133	0.151	0.141
10	0.205	0.201	0.207	0.204	0.221	0.261	**0.271**	**0.251**
15	0.311	0.298	0.276	0.295	0.344	0.358	0.325	0.342
20	0.342	0.371	0.386	0.366	0.415	0.421	0.401	0.412
25	0.418	0.411	0.421	0.416	0.503	0.508	0.498	0.503
Mean				0.28286				0.33006
SD				0.0054049				0.0057492
%RSD				1.909				1.515



Graph 3: Calibration curve of Caffeine at 273.0nm



Graph 4: Calibration curve of Quercetin at 373.0nm

3.5.1.1 Precision study

3.5.1.2 Repeatability

Table 7: Repeatability of Caffeine and Quercetin.

Concentration (µg/ml)	Absorbance of Caffeine	Absorbance of Quercetin
20	0.366	0.141
20	0.364	0.143
20	0.366	0.145
20	0.361	0.146
20	0.363	0.140
20	0.360	0.144
Mean	0.3633	0.14316
SD	0.002503	0.0023
% RSD	0.550	1.398

3.5.1.3 Intraday Precision

Table 8: Result of Intraday precision of Caffeine and Quercetin.

Conc. (µg/mL)	Day 1 Absorbance (Morning) at 273.0nm	Day 1 Absorbance (Afternoon) at 273.0nm	Day 1 Absorbance (Evening) at 273.0nm	Day 1 Absorbance (Morning) at 373nm	Day 1 Absorbance (Afternoon) at 373nm	Day 1 Absorbance (Evening) at 373nm
	Caffeine			Quercetin		
20	0.366	0.367	0.360	0.141	0.143	0.144
20	0.361	0.363	0.362	0.144	0.145	0.143
20	0.365	0.364	0.366	0.140	0.146	0.142
Mean	0.364	0.3646	0.3626	0.141	0.1446	0.143
SD	0.00264	0.00208	0.00305	0.00208	0.00152	0.001
%RSD	0.549	0.549	0.828	1.418	0.694	0.699
AVG % R.S.D	0.642			0.937		

3.5.1.4 Interday Precision

Table 9: Result of Interday Precision of Caffeine and Quercetin.

Conc. (µg/mL)	Day 1 Absorbance at 273.0nm	Day 2 Absorbance at 273.0nm	Day 3 Absorbance at 273.0nm	Day 1Absorbance at 373.0nm	Day 2Absorbance at 373.0nm	Day 3Absorbance at 373.0nm
20	0.366	0.366	0.360	0.142	0.141	0.145
20	0.365	0.361	0.367	0.145	0.140	0.145
20	0.363	0.362	0.365	0.143	0.144	0.146
Mean	0.3646	0.363	0.364	0.143	0.141	0.145
SD	0.00152	0.00264	0.00360	0.00152	0.00208	0.0005
%RSD	0.274	0.550	0.824	0.699	1.418	0.344
AVG %R.S.D	0.549			0.820		

3.5.1.5 Ruggedness

Table 10: Result of ruggedness of Caffeine and Quercetin.

Conc. (µg/mL)	Analyst-1 Absorbance	Analyst-2 Absorbance	Conc. (µg/mL)	Analyst-1 Absorbance	Analyst-2 Absorbance
	Caffeine			Quercetin	
20	0.367	0.366	10	0.142	0.144
20	0.366	0.363	10	0.144	0.145
20	0.364	0.362	10	0.146	0.147
Mean	0.365	0.363	**Mean**	0.144	0.145
SD	0.0015	0.0020	**SD**	0.002	0.001
% RSD	0.273	0.550	**% RSD**	1.388	0.689

3.5.1.6 Robustness

Table 11: Results showing robustness of Caffeine and Quercetin.

Conc. (µg/mL)	Absorbance at 10^0C	Absorbance at 25^0C	Absorbance at 45^0C	Conc. (µg/ml)	Absorbance at 10^0C	Absorbance at 25^0C	Absorbance at 45^0C
	Caffeine				Quercetin		
20	0.368	0.363	0.362	10	0.145	0.147	0.141
20	0.366	0.364	0.367	10	0.147	0.148	0.143
20	0.365	0.366	0.361	10	0.143	0.146	0.142
Mean	0.366	0.364	0.363	**Mean**	0.145	0.147	0.142
SD	0.00152	0.0015	0.00321	**SD**	0.002	0.001	0.001
% RSD	0.273	0.274	0.826	**% RSD**	1.379	0.680	0.704

3.5.2 LOD and LOQ of Quercetin and Caffeine

Table 12: Results showing LOD and LOQ of Caffeine and Quercetin.

Drug name	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
Caffeine	**273.0nm**	1.220	3.698
Quercetin	**373.0nm**	2.113	6.404

Table 13: Optical Characteristics and Validation Study of Drugs.

Parameters	Caffeine	Quercetin
Wavelength λ max nm	273.0nm	373.0nm
Beer's law limit µg/ml	5-25ug/ml	10-50ug/ml
Correlation coefficient (R^2)	0.9912	0.9945
Slope	0.0636	0.0643
Intercept	0.0146	0.0089
SD	0.0054	0.0057
% RSD	1.909	1.515
Precision		
Repeatability (%RSD)	0.550	1.398
Intraday (% RSD)	0.642	0.937
Interday (% RSD)	0.549	0.820

Ruggedness		
Analyst 1 (% RSD)	0.273	1.388
Analyst 2 (% RSD)	0.550	0.689
Robustness		
Temp. 10^0C (% RSD)	0.273	1.379
Temp. 25^0C (% RSD)	0.274	0.680
Temp. 45^0C (% RSD)	0.826	0.704
LOD (µg/ml)	1.220	2.113
LOQ (µg/ml)	3.698	6.404

DISCUSSION

The organoleptic properties, melting point determination, pH analysis, and FTIR studies of Caffeine and Quercetin confirmed their identity, purity, and compatibility for analytical method development. Both drugs showed characteristic physicochemical properties consistent with reported standards, while FTIR spectra verified the presence of important functional groups without evidence of chemical incompatibility. The developed UV–Visible spectrophotometric method demonstrated excellent linearity for both Caffeine and Quercetin within the selected concentration ranges, with high correlation coefficient values indicating reliable quantitative performance. Accuracy studies showed percentage recoveries close to 100%, confirming the validity of the method. Precision studies, including repeatability, intraday, and interday analysis, produced %RSD values below 2%, indicating excellent precision and reproducibility. Ruggedness studies confirmed that the method was operator-independent, while robustness studies revealed that small temperature variations did not significantly affect analytical performance. Overall, the developed method was found to be accurate, precise, robust, and suitable for routine quantitative analysis of Caffeine and Quercetin in pharmaceutical formulations.

4. CONCLUSION

The present study successfully developed and validated UV spectrophotometric methods for the estimation of caffeine and quercetin. Both compounds were clearly identified through organoleptic, solubility, melting point, pH, and FTIR studies, confirming their purity and physicochemical characteristics. Caffeine showed a λ max at 273.0nm and quercetin at 373.0nm, with an isobestic point at 263 nm, supporting their suitability for analysis. Within the Beer's law range of 5–25 µg/ml for caffeine (R^2 = 0.9912) and 10–50 µg/ml for quercetin (R^2 = 0.9945), the techniques showed satisfactory linearity. High reproducibility was demonstrated by precision studies with low %RSD values for repeatability (0.550% for caffeine, 1.398% for quercetin), intraday (0.642% and 0.937%), and interday (0.549% and 0.820%). Method dependability was further validated by robustness and robustness studies, which consistently showed %RSD values below 2% under various analysts and temperature circumstances. With LOD and LOQ values of 1.220µg/ml and 3.698µg/ml for caffeine and 2.113µg/ml and 6.404µg/ml for quercetin, respectively, the method's sensitivity was likewise good. All things considered, the new UV spectrophotometric techniques are straightforward, quick, economical, and trustworthy. These techniques are appropriate for routine quantitative analysis of caffeine and quercetin in pharmaceutical and quality control applications because all validation parameters adhere to ICH requirements.

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