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SPECTROPHOTOMETRIC DETERMINATION OF QUERCETIN USING MICELLES OF CETYLTRIMETHYLAMMONIUM BROMIDE IN A LOW RATIO METHANOL-WATER MIXTURE[1]

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A simple and achievable UV-Vis spectrophotometric method for the micro-quantitative determination of quercetin has been developed and validated. This method relies on the formation of supramolecular assemblies of quercetin (QR) and the cationic surfactant cetyltrimethylammonium bromide (CTAB) in a 5 % methanolic aqueous solution. In this solvent medium, CTAB in the presence of QR has a critical micelle concentration of $1.2 \cdot 10^{-3}$ mol l^{-1} at 24 °C, determined through conductometry. Important analytical parameters such as wavelength, composition of methanol-water mixture, CTAB concentration (c_{CTAB}), and pH were optimized. Under the optimum experimental conditions (λ = 397 nm, 5 % methanol as solvent, $c_{CTAB} = 2.0 \cdot 10^{-3}$ mol l^{-1}, and pH = 6.0), Beer's law was valid for QR concentrations up to 16.9 μg ml^{-1}. The Ringbom optimum QR concentration range was 1.0 – 16.9 μg ml^{-1}. The method sensitivity was $2.03 \cdot 10^4$ l mol^{-1} cm^{-1} (the molar absorptivity) and 0.13 μg ml^{-1} (limit of detection). The applicability of the proposed method for quantifying QR in pharmaceutical formulations was demonstrated. Furthermore, the proposed UV-Vis spectrophotometric method was successfully applied to the reliably assay of QR, even in the presence of vitamin C.

Keywords: spectrophotometry; quercetin; cetyltrimethylammonium bromide micelles; dietary supplements

СПЕКТРОФОТОМЕТРИСКО ОПРЕДЕЛУВАЊЕ НА КВЕРЦЕТИН СО КОРИСТЕЊЕ МИЦЕЛИ ОД ЦЕТИЛТРИМЕТИЛАМОНИУМ БРОМИД ВО СМЕСА СО МАЛ ОДНОС НА МЕТАНОЛ-ВОДА

Развиен е и валидиран едноставен и остварлив UV-Vis спектрофотометриски метод за микроквантитативно определување на кверцетин. Овој метод се потпира на формирање супрамолекуларни здружувања на кверцетин (QR) и катјонски сурфактант цетилтриметиламониум бромид (CTAB) во 5 % метанолен воден раствор. Во овој растворувач, CTAB во присуство на QR има критична мицелна концентрација од $1{,}2 \cdot 10^{-3}$ mol l^{-1} на 24 ºC, одредена преку спроводливост. Извршена е оптимизација на важните аналитички параметри како што се брановата должина, составот на смесата метанол-вода, концентрацијата на CTAB (c_{CTAB}) и pH. Под оптимални експериментални услови (λ = 397 nm, 5 % метанол како растворувач, $c_{CTAB} = 2{,}0 \cdot 10^{-3}$ mol l^{-1} и pH = 6,0) важи Беровиот закон за концентрации на QR до 16,9 μg ml^{-1}. Оптималниот опсег на концентрација според Ringbom на QR беше 1.0 – 16.9 μg ml^{-1}. Чувствителноста на методот беше $2.03 \cdot 10^4$ l mol^{-1} cm^{-1} (моларна апсорпција) и 0.13 μg ml^{-1} (граница на детекција). Докажана е

[1] Dedicated on the occasion of the Golden Jubilee of the *Macedonian Journal of Chemistry and Chemical Engineering*

применливоста на предложениот метод за квантифицирање на QR во фармацевтските формулации. Покрај тоа, предложениот UV-Vis спектрофотометриски метод беше успешно применет за вородостојна анализа на QR, дури и во присуство на витамин C.

Клучни зборови: спектрофотометрија; кверцетин; мицели на цетилтриметиламониум бромид; додатоци во исхраната

1. INTRODUCTION

Among all flavonoids, a large group of bioactive polyphenolic compounds widely present in plants, quercetin (3,3',4',5,7-pentahydroxyflavanone, Fig. 1) stands out as a unique flavonoid that has attracted considerable interest in the scientific community over the past decade.[1] Since flavonoids, including quercetin, exhibit diverse biological activities such as antioxidative, anti-inflammatory, antidiabetic, anticancer, and antiviral properties,[2] current research trends focus on their health benefits for humans. Particularly noteworthy are the potential therapeutic applications of some flavonoids and their metal complexes against infectious diseases.[3] This is especially relevant in the light of the ongoing COVID-19 infections caused by the novel SARS-CoV-2 virus.[4–6]

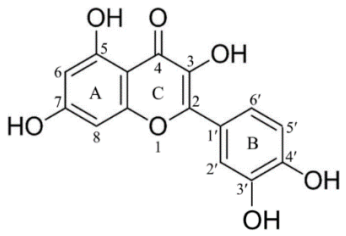

Fig. 1. Chemical structure of quercetin ($C_{15}H_{10}O_7$, 3,3',4',5,7-pentahydroxyflavanone)

Quercetin (hereinafter referred to as QR) is the aglycone form of flavonoid derived from plants.[7] It is abundantly found in fruits, vegetables, and plant-based beverages such as apples, cherries, citrus fruits, onions, broccoli, tea, and red wine. Quercetin is present not only in its free form but also as glycosides, with rutin (quercetin-3-O-rutinoside, 3′,4′,5,7-tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone) being the dominant compound among them.

Quercetin, like other flavonoids found in fruits and leafy vegetables, exhibits a significant array of biological/pharmacological activities,[1,8-10] most notably its chelating activity with metal ions.[11] Due to the presence of several adjacent hydroxyl groups in its benzene rings (Fig. 1), QR easily chelates metal ions, primarily iron or copper ions, thereby preventing them from participating in oxidative processes. In addition to metal-binding capabilities, the high *in vitro* antioxidant activity of QR results from its ease of oxidation and the ability of its phenolic residue to delocalize generated radicals. As a result, QR stabilizes the cell membrane, inhibits the aging processes in tissues such as skin, cornea, and myocardium, and offers various health benefits, including protection against various diseases such as osteoporosis, lung cancer, and cardiovascular disease.[7,12] Apart from its antioxidative properties, QR also exhibits antiviral activity,[4,13,14] inhibiting the replication and infectivity of certain RNA and DNA viruses, such as several members of the *Coronaviridae* family. QR also influences various host cell signaling processes, inducing of gene transcription factors and cytokines secretion.[15]

Given QR's significant properties, many dietary supplements and pharmaceutical preparations contain QR either individually or in combination with other flavonoids (mostly rutin) or with ascorbic acid (AA). There is also evidences that QR in combination with vitamin D, estradiol, antivirals, or other drugs may synergistically provide additional therapeutic options for preventing and treating various phases of complex diseases.[16]

Therefore, a simplified analytical technique for the QR determination is required. Moreover, the ongoing development of new methods for flavonoid determination has made steady progress due to increased interest in assessing the antioxidant activity of numerous substances and antioxidant status in humans. QR frequently serves as a reference substance in these assays for determining the antioxidant activity of various samples.

In recent years, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have emerged as leading instrumental methods for separating, identifying and quantifying QR in extracts from plants, foods, and beverages. For determining this compound in pharmaceuticals and dietary supplements with relatively simple composition, spectroscopic and electrochemical methods are suitable.[17–20] However, despite their sensitivity and efficiency, some of the methods involve time-consuming extraction procedures or

expensive instrumentation and sensing materials. Additionally, some of them generate excessive waste and fail to meet green chemistry criteria. Therefore, there is a need for a simple and rapid method suitable for routine use, such as the direct spectrophotometric method, for determining QR in pharmaceutical dosage forms and food.[21,22]

On the other hand, an important issue is the mode of flavonoid interaction with appropriate target proteins at the molecular level, which is still unknown. Related to those investigations, self-assembled cationic surfactants like cetyltrimethylammonium bromide have been used to simulate cells and proteins in living systems.[23,24] In such a system, QR properties such as hydrophobicity and planarity with other flavonoids, and consequently, its stability, can be moderate due to the sensitivity of quercetin′s polyphenolic structure to environmental changes. Recently, a spectrofluorimetric method based on the formation of stable supramolecular complexes between QR and micelles of some surfactants (such as cetyltrimethylammonium bromide, sodium dodecyl sulphate, and triton X-100) in aqueous media was proposed.[25]

In this paper, we utilized surfactant micelles (cetyltrimethylammonium bromide, hereinafter referred to as CTAB) to enhance the spectrophotometric quantification of quercetin. Therefore, this study aimed to develop and validate a straightforward spectrophotometric method for QR determination based on its interaction with CTAB by simply selecting optimum parameters such as wavelength, solvent type, and pH. Additionally, to assess the usefulness of the method for QR determination in pharmaceutical formulations, we also investigated the effects of possible interfering substances, particularly ascorbic acid.

Furthermore, while other methods used for QR determination may offer greater sensitivity and efficiency than the spectrophotometric method,[26] they are often costly and require complex sample pretreatment not necessary for routine analysis of the QR content in pharmaceuticals. Therefore, the procedure proposed in this work eliminates an extraction step using organic solvents. It also reduces the time required for full analysis, making it a suitable alternative to the existing analytical tools for quantifying QR in bulk and pharmaceutical formulations.

2. EXPERIMENTAL

2.1. *Chemicals and solutions*

All the chemicals used were of analytical reagent grade. Cetyltrimethylammonium bromide, naringin, and naringenin were obtained from Sigma-Aldrich (St. Louis, United States). Quercetin dihydrate, morin dihydrate, methanol, HCl, and NaOH were purchased from Merck (Darmstadt, Germany). Rutin was purchased from Fluka (Buchs, Switzerland), while ascorbic acid and magnesium ascorbate came from Sigma-Aldrich (St. Louis, United States). *Quercetin Nettle Complex* capsules were obtained from Magnifood, Terranova, Greece. Deionized water with a specific resistance of 18 MΩ cm^{-1} (Milli-Q, Millipore, Bedford, MA, USA) was used as the solvent throughout.

The standard stock solutions of QR and CTAB were prepared at least one day before starting the experiments to minimize possible time-dependent effects. A CTAB stock solution (c_{CTAB} = $5 \cdot 10^{-2}$ mol l^{-1}) was prepared in both deionized water and 5 % methanol at room temperature by weighing an appropriate mass of CTAB (0.9111 g), quantitatively transferring into a 50 ml volumetric beaker, and dissolving in 40 ml of either water or 5 % methanol. The mixture was gently stirred with a glass rod to avoid foam formation. Subsequently, the solution was transferred into a 50 ml volumetric flask and diluted to volume with water or 5 % methanol.

The standard stock solution of QR (c_{QR} = $6 \cdot 10^{-4}$ mol l^{-1}) was prepared by dissolving 0.0507 g of QR in 250 ml of 50 % methanol. The stock solutions of both QR and CTAB standards were prepared in volumetric glass flasks wrapped with aluminum foil to protect them from daylight and stored in a refrigerator until needed. These standards were further diluted to produce the standard working solutions of the QR-CTAB system for spectrophotometric measurement.

The pH of the solutions was adjusted using HCl/NaOH according to the procedure previously described in detail.[27] For the interference study, appropriate amounts of possible interfering substances, such as ascorbic acid and some flavonoids, were dissolved in 5 % methanol. After further dilution with the same solvent, the final concentrations for the interference study were obtained.

2.2. *Apparatus*

Spectrophotometric measurements were performed using a UV-Vis Spectrophotometer Beckman DU-650 (Fullerton, Ca, USA) with a 1 cm quartz cuvette. A methanolic aqueous solution (5 %, 10 %, 15 %, or 20 % v/v) at the same pH as the QR-CTAB system (solution) was used as a blank. For pH measurements, a pH-meter (pHM-82 Radi-

ometer, Copenhagen), with an accuracy of ± 0.001 pH, equipped with the combined electrode (No. CW. 733 Series No. 35162, Russel) was used. To determine the critical micellar concentration (CMC) of 5 % methanolic aqueous solution of CTAB in the presence of QR (pH = 6, t = 25 °C), conductivity measurements were performed using a digital conductivity meter HI8820N (Hanna instruments, Portugal) with the matching HI7684W probe that uses the 4-ring method. The CMC value was determined using the conventional method (Williams' method).[28] The temperature was controlled within ± 0.2 °C by circulating a water thermostat (Series U, MLW Freital, Germany). An ultrasonic bath (L.U.5.7 Fungilab, S.A, Spain) was used to dissolve the samples.

2.3. *Conductometric measurement*

The conductivity-CTAB concentration data were obtained for the series of CTAB solution systems in 5 % v/v methanol by increasing the CTAB concentration from 0.5 mM to 2.0 mM, while keeping the QR concentration constant (c_{QR} = $5.0 \cdot 10^{-5}$ mol l^{-1}) at constant temperature (25.0 °C). Fresh stock solutions of CTAB in water (c_{CTAB} = $2.0 \cdot 10^{-3}$ mol l^{-1}) and QR in 50 % v/v methanol (c_{QR} = $4.0 \cdot 10^{-4}$ mol l^{-1}) were used for preparing standard solutions for measuring specific conductivity. These solutions were prepared one day before conductivity measurements to minimize possible time-dependent effects. The preparation was as follows: 10 ml of $4.0 \cdot 10^{-4}$ mol l^{-1} QR solution, prepared in 50 % v/v methanol, was transferred to a 100 ml volumetric flask. Then, a certain volume of the CTAB stock solution was added to achieve previously defined concentrations, and filled up to the mark with deionized water. These measurements were carried out in an 80 ml glass vessel with a tightly closed cover that had openings for the thermometer, the combined electrode, and the conductometric cell.

2.4. *Spectrophotometric measurement*

2.4.1. *Determination of QR in pure form*

To obtain the calibration curve for QR determination, a series of standard stock solutions of the QR-CTAB system was used, in which QR concentration was varied from $1.0 \cdot 10^{-6}$ mol l^{-1} to $6.0 \cdot 10^{-5}$ mol l^{-1}, while the CTAB concentration was kept constant (c_{CTAB} = $2.0 \cdot 10^{-2}$ mol l^{-1}). Standard solutions were prepared in 25-ml volumetric flasks by mixing appropriate volumes of the QR standard stock solution (V_1), 50 % methanol (V_2), and aqueous stock solution of the CTAB (V_3), following this order. To obtain the final solutions of the required molar concentrations in 5 % methanol, it was necessary that $V_1 + V_2$ = 2.5 ml and V_3 = 22.5 ml before being filled up to the mark with 5 % methanol. These buffered standard solutions (pH = 6) were allowed to stabilize for at least 15 min, and then the absorbance was measured in duplicate at 397 nm against a 5 % methanol solution at pH = 6 as a blank.

2.4.2. *Determination of QR in pharmaceutical formulation*

To determine the amount of QR in the pharmaceutical dosage form, the contents of ten capsules were mixed and weighed accurately, and the average mass of one capsule (0.5618 g) was transferred to a 1000-ml volumetric flask. This capsule content was dissolved in about 500 ml of 50 % methanol and left for 20 min for complete dispersion. After sonication and shaking the mixture for 40 min, the dispersion was filtered through Whatman No. 1 filter paper. The residue was washed well with 50 % methanol for complete drug recovery, and the volume was completed with the same solvent. Then, a 1.4 ml aliquot was transferred to a 25-ml volumetric flask and mixed with 1.1 ml of 50 % methanol as well as 25 ml of aqueous CTAB solution. The whole procedure of sample preparation was repeated three times, and the amount of QR in the sample was determined as the average of three measurements. The recovery of the drug was computed from the corresponding regression equation. According to the declaration, two capsules contain 400 mg of QR and 150 mg of vitamin C (as magnesium ascorbate).

3. RESULTS AND DISCUSSION

3.1. *Optimization of spectrophotometric experimental conditions*

Once it was shown that the interaction between flavonoids and surfactants promotes absorbance and confers enough stability to the flavonoids during their spectrometric quantification time due to the supramolecular interaction with surfactant micelles,[24,25,29–32] the spectrophotometric determination of QR under particular experimental conditions was carried out. Preliminary experiments were performed to find out the optimized operative conditions and several analytical performance characteristics were evaluated for the quantifica-

tion of QR in both pure form and pharmaceutical preparation. The optimized experimental parameters included wavelength, CTAB concentration, solvent composition, and pH.

In the UV-Vis spectrum of QR (curve 1, Fig. 2), two major absorption maxima are observed: one at about 250 nm and the other at about 365 nm, commonly referred to as band II and band I, respectively, which are in excellent agreement with the literature.[24] Absorption band II may be considered to have originated from π–π^* electron transitions in the A + C ring benzoyl system of QR, while band I is associated with the absorption of the cinnamoyl system (B + C ring) (Fig. 2).[18,33,34] With the addition of CTAB in different concentrations (below, around, as well as the above CMC) to the 5% methanolic aqueous solution of QR (c_{QR} = $5\cdot10^{-5}$ mol l^{-1}), an enhancement in the intensity of absorption bands was observed (Fig. 2). Namely, the peak intensity of the bands increased with gradual increasing of CTAB concentration. Additionally, the absorption peaks of band II and band I both show distinct changes. As seen from Figure 2, there is a red shift of bands; the presence of CTAB causes a bathochromic shift of about 6 – 35 nm from the original band I and about 7 – 25 nm from the original band II in the absence of CTAB (curves 2–7, Fig. 2). Specifically, the CTAB pre-micelles make the QR absorption peak at about 365 nm shift to a longer wavelength with decreased intensity (curves 2–4, Fig. 2), and CTAB spherical micelles cause the absorption peak intensity to increase (curves 5–7, Fig. 2) in comparison to the CTAB pre-micelles, while both CTAB premicelles and micelles (curves 2–5) decrease the peak intensity of the QR original band II, with the absorption maximum shifted to the longer wavelength. It is obvious that the best conditions for QR quantification relate to the λ = 397 nm and c_{CTAB} = $2\cdot10^{-3}$ mol l^{-1}. This optimal CTAB concentration is about 1.7 times higher than the respective CMC ($1.2\cdot10^{-3}$ mol l^{-1}), obtained by plotting the graph between specific conductance and CTAB concentration in 5 % v/v methanol solution containing fixed QR concentration (c_{QR} = $5\cdot10^{-5}$ mol l^{-1}) and ensures the presence of micelles as well as the predominance of the QR-CTAB complex in the system. Additionally, in the presence of CTAB micelles, the observed peak at λ = 397 nm does not change in intensity over time. Thus, the use of CTAB surfactant provides sufficient stability to QR during its spectrophotometric quantification time.

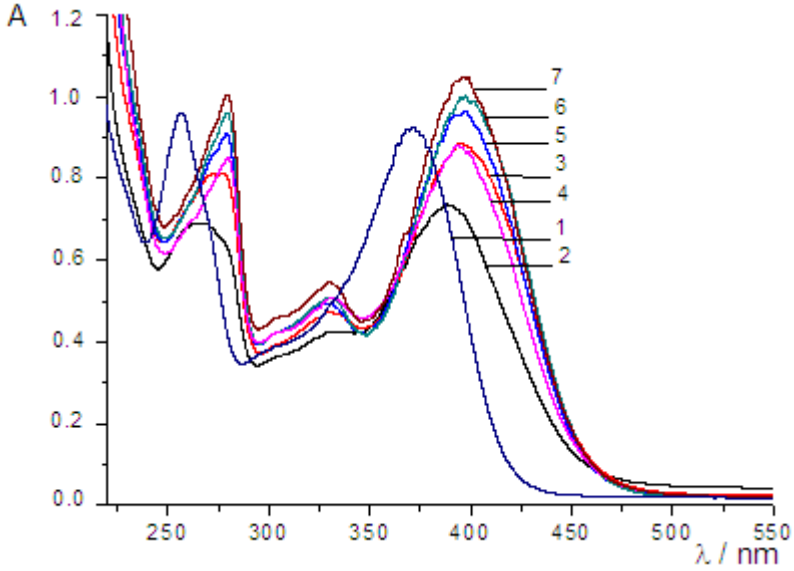

Fig. 2. UV-Vis absorption spectra of 5 % methanol solutions of QR ($5\cdot10^{-5}$ mol l^{-1}) in the absence (1) and presence of different CTAB concentrations (in mmol l^{-1}): 0.5 (2), 0.9 (3), 1.0 (4), 1.5 (5), 2.0 (6), and 6.0 (7) against a 5 % methanol solution at pH = 6 as a blank

The influence of the medium composition on the absorption spectra of QR (c_{QR} = $5\cdot10^{-5}$ mol l^{-1}) in the presence of CTAB (c_{CTAB} = $2\cdot10^{-3}$ mol l^{-1}) was investigated (Fig. 3). It was observed that the existence of the CTAB micelles in different solvent compositions (methanol-water mixtures containing a volume fraction of methanol in the range of 5 – 20 % volume fraction) exerted nearly no influence

on band II of quercetin. In contrast, the increase in methanol percentage caused a small hypochromic shift of band I with a decrease in the band peak intensity. Therefore, the best conditions (the higher absorption peak intensity) relate to the 5 % methanol.

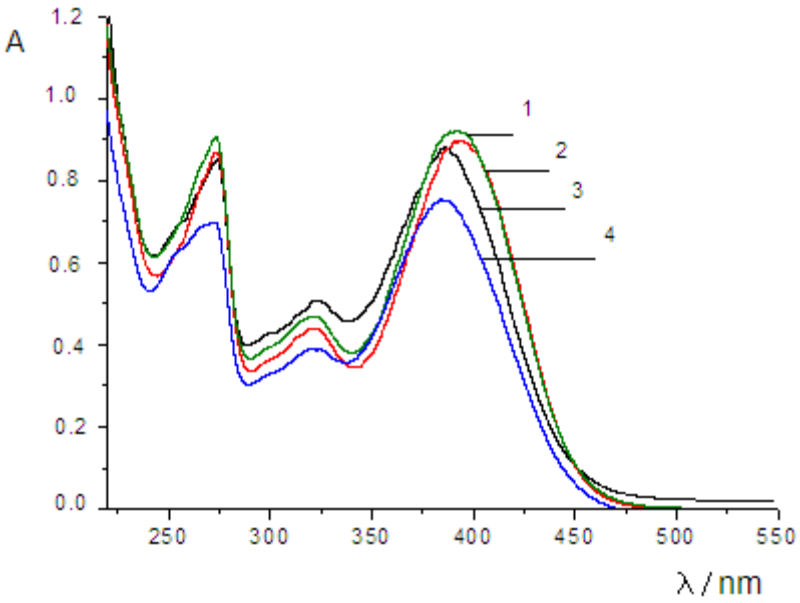

Fig. 3. UV-Vis absorption spectra of QR (c_{QR} = 5·10^{-5} mol l^{-1}) in the presence of CTAB in different methanol-water mixtures (v/v): 5 % (1), 10 % (2), 15 % (3), and 20 % (4); pH = 6.0; c_{CTAB} = 2·10^{-3} mol l^{-1}

In the end, keeping all conditions unchanged, the influence of pH within the pH range of 3.0 – 8.0 on the absorbance of 5 % methanolic aqueous solutions of QR (c_{QR} = 4·10^{-5} mol l^{-1}) in the presence of CTAB (c_{CTAB} = 2·10^{-3} mol l^{-1}) was studied. The change in acidity induced a bathochromic shift of about 7 – 40 nm from the original band I in the absence of CTAB with a change of the pH.

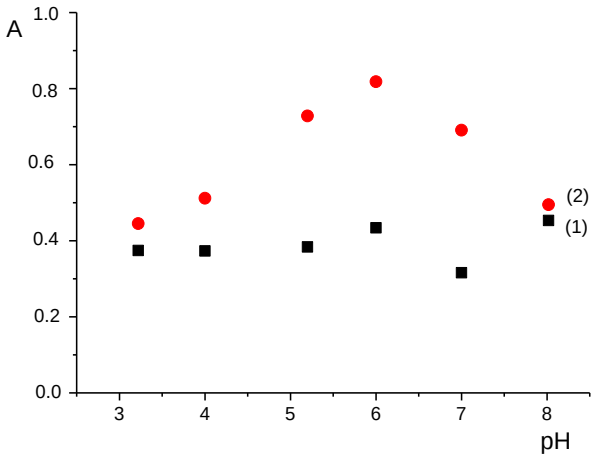

Fig. 4. The absorbance of 5 % methanolic solutions of QR (c_{QR} = 4·10^{-5} mol l^{-1}) in the absence (1) and the presence (2) of CTAB micelles at various pH values; λ = 397 nm and c_{CTAB} = 2·10^{-3} mol l^{-1}

As is well known, the degree of dissociation of the functional groups in the QR, *i.e.*, dynamic equilibria of ion-molecular forms of QR changes, and consequently, the interaction between the QR and CTAB micelles, is also modified. However, the best conditions (the higher absorbance) relate to pH 6.0 (Fig. 4). In addition, it was found that the absorption peak intensity of QR in the presence of CTAB is higher than the absorbance of QR without CTAB for each pH in the examined pH interval. Nevertheless, CTAB micelles promote both QR absorbance and time stability, and pH 6.0 was chosen as the optimal pH for QR quantification using absorption spectrophotometry.

3.2. *Analytical features*

Under the optimal experimental conditions for QR determination, which were 5 % methanol as solvent, c_{CTAB} = 2·10^{-3} mol l^{-1}, and pH = 6.0, the absorbances of working solutions prepared by addition of the constant CTAB concentration (c_{CTAB} = 2·10^{-3} mol l^{-1}) to the a series of diluted standard stock solutions of QR (1·10^{-6} ≤ c_{QR} ≤ 6·10^{-5} mol l^{-1}) were measured at 397 nm against the blank solution (5 % methanol at pH = 6.0). Absorbance peak intensities were recorded at a fixed time point (after 20 min of QR and CTAB mixing and buffering). A plot of absorbance (A) against the QR concentrations

(c_{QR}) provided a calibration graph that was fitted by the least-squares method.

To ensure that the proposed method provides reliable analytical data, it is necessary to evaluate the optimized procedure and check it against important factors such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision.

The calibration curve exhibited excellent linear behavior over the concentration range from 0.68 μg ml^{-1} to 16.90 μg ml^{-1} (about 1.5 orders of magnitude) with a correlation coefficient of 0.9997. Analytical features of the calibration curve and analytical figures of merit for the QR determination, which included the Beer-Lambert's law limit, Ringbom optimum concentration range, molar absorptivity, Sandell's sensitivity, parameters of the regression equation (intercept, slope, standard deviation of the intercept and slope as well as the correlation coefficient), limit of detection (LOD), and limit of quantification (LOQ) are summarized in Table 1.

The optimum concentration range for accurate QR determination, as established from Ringbom plot, *i.e.*, a plot of (1–*T*) *versus* log c_{QR} (where *T* and c_{QR} is the transmittance and the QR concentration that obeys Beer's law, respectively), is 1.00 – 16.90 μg ml^{-1}. The relative analysis error, as calculated from the slope of the linear segment of the obtained curve, is 0.035 (the slope of the Ringbom plot is 0.658; the ratio between the relative error in QR concentration and photometric error is 3.50 for $\Delta p = 0.01$).

To prove the precision of the proposed method, three different concentrations of QR in bulk drug (1, 5, and 10.0 μg ml^{-1}) within Beer's law limits were tested by replicate analysis (four independent measurements).

T a b l e 1

Performance data and analytical parameters for quercetin determination by proposed spectrophotometric method

Parameter	Analytical features
Analytical wavelength /nm	397
Beer's law limits /μg ml^{-1}	0.68 – 16.90
Ringbom optimum range /μg ml^{-1}	1.00 – 16.90
Molar absorptivity /10^4 l mol^{-1} cm^{-1}	2.03
Sandell′s sensitivity /μg cm^{-2}	0.017
LOD[a] /μg ml^{-1}	0.14
LOQ[b] /μg ml^{-1}	0.42
Regression equation (A = a + b c_{QR})[c]	
Intercept (a)	0.009
Slope (b) /μg^{-1} ml cm^{-1}	0.060
Standard deviation of intercept (S_a)	0.0025
Standard deviation of slope (S_b)	0.0003
Coefficient of regression (R^2)	0.9997
Standard deviation (n = 9)	0.0051

[a] Limit of detection[35,36] calculated from 3.3 × S_a/b (S_a is the standard deviation of intercept).
[b] Limit of quantification[35,36] expressed as 10 × S_a/b.
[c] c_{QR} is the QR concentration in μg ml^{-1}.

The obtained results, summarized in Table 2, confirmed the precision of the proposed method. The RSD values, which are less than 3 % for the three different concentration levels studied, indicate the high repeatability of the method. The lowest RSD value (1.1 %) was obtained for c_{QR} = 10.0 μg ml^{-1}, so the sample solution containing this QR concentration was used for further quantitative sample analysis, which was performed by four replicate absorbance measurements of a sample solution under optimal experimental conditions.

T a b l e 2

Precision of proposed spectrophotometric method and recovery of quercetin in bulk drug and capsules

Sample	Taken / μg ml^{-1}	Found ±SD / μgml^{-1}	RSD*[a] %	RCV %
QR in bulk	1.0	1.03 ± 0.03	3.8	102.7
	5.0	5.17 ± 0.05	2.5	103.3
	10.0	9.91 ± 0.07	1.1	99.1
QR in capsules	10.0	9.92 ± 0.08	2.0	99.1

[a] RSD$^* = \frac{t\,\mathrm{RSD}}{\sqrt{n}}$, where t-value is the critical value at 95 % confidence limit for three degrees of freedom

To provide additional support for the accuracy of the proposed method, a standard addition method was used. Accuracy was assessed as recovery value (RCV) calculated as: [total QR concentration measured after standard addition – QR concentration in the formulation sample/ QR concentration added] × 100. The mean recovery of QR from pharmaceutical formulation after spiking with

1.4, 4.7, and 9.5 μg ml^{-1} of additional QR standard was 99.2 % with mean RSD of 2.6 % (Table 3). Because the recoveries of QR added to the capsules were close to 100 % (Table 3), it may be assumed that there was no potential interference from ascorbic acid and additives/excipients in the capsule formulation.

T a b l e 3

Recovery data for quercetin spiked to pharmaceutical formulation (capsules)

Pharmaceutical formulation	Added / μg ml^{-1}	Found / μg ml^{-1}	Recovery* %	Average recovery %	RSD %
Capsules	0.0	4.0	–		
	1.4	5.4	98.6	99.2	2.6
	4.7	8.6	97.9		
	9.5	13.6	101.1		

*Average of three determinations

3.3. *Method selectivity*

The selectivity of the proposed method was demonstrated by similar absorption profiles of the working standard and pharmaceutical formulation (Fig. 5) under optimal experimental conditions, suggesting the same identity of the samples. Both the standard and the sample exhibited absorption at 397 nm under selected assay conditions. The presence of the antioxidant agent, vitamin C in the form of L-ascorbate (magnesium ascorbate), which coexists with QR in capsules, did not affect the spectrophotometric analysis of the QR in the UV-Vis spectral region (Fig. 5).

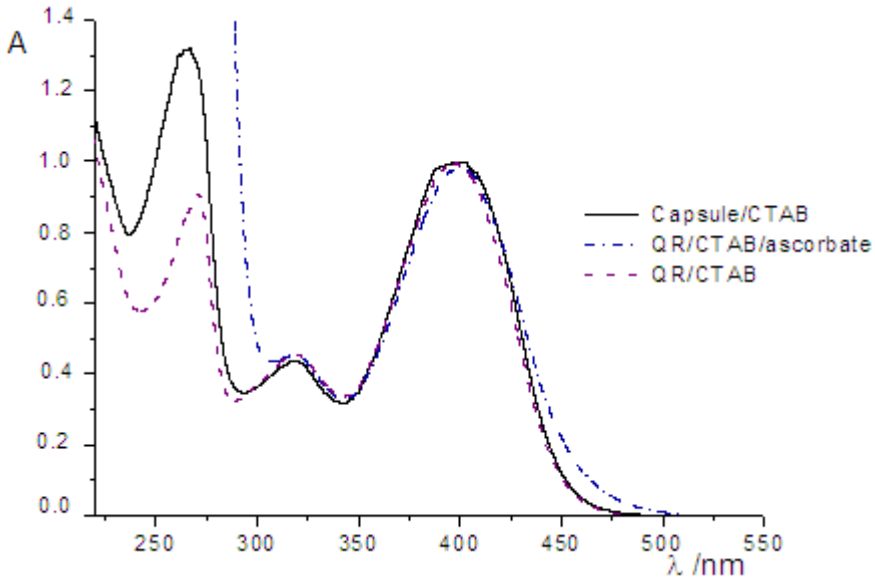

Fig. 5. Overlap of UV-Vis absorption spectra of solutions of a standard mixture of QR and CTAB (QR/CTAB), pharmaceutical formulation and CTAB (capsules/CTAB), as well as QR, CTAB, and magnesium-ascorbate (QR/CTAB/ascorbate); all were in 5 % methanolic aqueous solution at pH = 6; $c_{QR} = 5 \cdot 10^{-5}$ mol l^{-1}, $c_{CTAB} = 2 \cdot 10^{-3}$ mol l^{-1} and $c_{ascorbate} = 1.5 \cdot 10^{-5}$ mol l^{-1}

To broaden the applicability of the proposed spectrophotometric method for QR quantification, the effects of some possible compounds that can provoke interference, such as molecules that could compete with quercetin in the CTAB micelles, including morin, rutin, naringin, and naringenin, were studied. The extent of interference from these structurally related compounds, as well as vitamin C (either ascorbic acid or L-ascorbate) as a relevant concomitant substance, were determined by measuring the absorbance of mixtures containing fixed concentrations of both QR ($1.0 \cdot 10^{-5}$ mol l^{-1} or $5.0 \cdot 10^{-5}$ mol l^{-1}) and CTAB ($2.0 \cdot 10^{-3}$ mol l^{-1}) with various concentrations of possible interferents

under optimum experimental conditions. An error of ± 3 % in the absorbance readings was treated as tolerable. The compounds examined should not interfere below tolerable ratios (taken as the concentration of interferent and concentration of QR ratio). For QR determination, the following compounds, when present in mole concentration for which a tolerable ratio is shown in brackets, do not affect absorbance measured: naringenin [6], naringin [0.7], morin [0.5], and rutin [0.01]. It is evident that the addition of all examined structurally related compounds strongly affects the absorbance measured. Among them, rutin is a major interferent and produces more than 5 % error in absorbance reading, even if it is present in a very low concentration (> 0.06 μg ml^{-1}) in a mixture with QR. Therefore, the essential constraint of the proposed method is a strong interference with other bioflavonoids (regardless of the presence of an O-glucosyl group in their molecular structure) when they present in concentrations greater than 16.3 μg ml^{-1} (naringenin), 4.1 μg ml^{-1} (naringin), 1.5 μg ml^{-1} (morin), and 0.06 μg ml^{-1} (rutin). Consequently, the proposed spectrophotometric method may be suitable for QR quantification in samples such as pharmaceutical formulations that either contain low concentrations of other bioflavonoids as in our case, or do not contain them at all.

On the other hand, an error of not more than 3 % was observed by up to a 5-fold molar excess of vitamin C. Therefore, it was determined that L-ascorbate does not significantly interfere with absorbance readings, even when present at concentrations up to 0.3 times that of QR in the sample formulation. Additionally, as vitamin C concentration in typical dietary supplements or nutraceuticals is generally not more than about two times higher than QR,[37] the proposed spectrophotometric method has the potential to be useful for determining the QR assay in the presence of vitamin C.

3.4. *Analysis of capsules formulation*

The validity of the proposed spectrophotometric method for the sample assay was tested on the marketed formulation, Quercetin + Vitamin C. It was determined that each capsule contained 193.6 ± 2.2 mg of QR (RSD = 1.2 %), whereas the QR content, according to the producer's declaration, was 400 mg per two capsules. The recovered drug content was 96.0 %, which suggests satisfactory agreement between the results obtained by the proposed methods and the label claim. Furthermore, analytical recovery studies (standard addition method) carried out to check the method validity (Table 2) showed good QR recovery, suggesting no interference from formulation additives/excipients. Additionally, the overlap of the absorption spectra of the QR standard solution and sample solution (capsules) indicated that the additives/excipients do not interfere with the sample absorbance reading in the proposed spectrophotometric analysis (Fig. 5).

In comparison to the analytical performances of some previously reported spectrophotometric methods (Table 4), the proposed method for the QR determination has a lower limit of detection and comparable or higher sensitivity than some of them. Nevertheless, the results of the developed method disclose that the present approach is a sensitive and accurate technique that requires neither sophisticated instruments nor sample pretreatment and may be considered as a suitable alternative to the existing methods.

T a b l e 4

Comparison of the analytical performance of previously reported spectrophotometric methods with the proposed method in this work for QR quantification

Reagent	λ_{max} (nm)	Beer's law / μg ml^{-1}	Molar absorptivity / l mol^{-1} cm^{-1}	LOD / μg ml^{-1}	Reference
N-bromosuccinimide	510	2.5–30	$8.14 \cdot 10^3$	_	(38)
–	370	1.0–12.0	/	0.76	(21)
Potassium titanyloxalate	430	0.85–16.9	$2.50 \cdot 10^4$	0.67	(39)
Cu(II)	458.5	0.2–1	$2.1 \cdot 10^4$	0.067	(40)
Al(III)	425	/	$2.52 \cdot 10^4$	0.2	(41)
Zn(II)	363	0.1-6.0	/	0.03	(42)
–	256	10–50	/	0.145	(43)
CTAB	397	0.68–13.53	$2.03 \cdot 10^4$	0.14	This work

4. CONCLUSION

In this study, a simple, inexpensive and sensitive spectrophotometric method based on QR interaction with CTAB micelles in a nearly aqueous medium has been developed for determining QR in bulk drug and pharmaceutical preparations (capsules). This method does not require any sophisticated apparatus or pretreatment of the samples. Additionally, there was no interference from vitamin C in the examined product. Thus, the lack of additional extraction or separation procedures is a further advantage. The proposed spectrophotometric method's broad applicability is slightly limited by interference with structurally related compounds such as rutin, morin, naringin, and naringenin in concentrations higher than 0.06, 1.5, 4.1, and 6.3 μg ml^{-1}, respectively. Hence, the proposed method is suitable for determining QR in pharmaceuticals containing sufficiently low concentrations of these compounds. Given the different requirements for analyzing samples with widely varying QR concentrations (pharmaceutical versus natural samples), the proposed spectrophotometric method can be easily used for the routine analysis of samples in which quercetin is a major flavonoid, and it can meet the needs of conventional analytical laboratories that do not possess highly sophisticated equipment.

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