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SPECTROPHOTOMETRIC QUANTIFICATION OF QUERCETINE USING A MICELLE SYSTEM

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ABSTRACT

The current research provides the development of the new spectrophotometric method for the simple and affordable quercetin quantification. Proposed method based on formation of supramolecular assembles of quercetin (Q) and cetyl trimethylammonium bromide (CTAB). Under selected experimental conditions ($\lambda = 378$ nm, $c_{CTAB} = 2$ mM and T = 25 °C), Beer's law was obeyed in the quercetin concentration ranged $2 \times 10^{-6} – 8 \times 10^{-5}$ mol dm^{-3}. The method sensitivity was 2.1×10^4 dm^2 mol^{-1} (the molar absorptivity) as well as 1×10^{-6} mol dm^{-3} (the limit of detection). The method applicability to the direct Q determination in a pharmaceutical formulation (Quercetin+C capsules, Twinlab) was demonstrated.

INTRODUCTION

Among all the flavonoids, which display a significant array of biological and pharmacological activities (antioxidative, antinflamatory, antimicrobial, anticancerogeneous, etc.), quercetin (3,3′,4′,5,7-pentaxydroxyflavone) is commonly found in food and plant based beverages such as onions, apples, tea and red wine. Since quercetin possesses mentioned very important properties, and thus it is distributed in many pharmaceutical preparations, their quantification is of a significant importance.

Besides spectrophotometric method based on colored complexing reaction of Q with many different inorganic reagents [1], various methods have been described for the Q determination. However, some of the methods are time consuming since quercetin stability is time or pH dependent, while some of them require expensive instrumentation or sensing materials. As a result, simplified method for routine use, such as direct spectrophotometric method [2] is required for the Q determination in food and pharmaceutical dosage forms.

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It is well know that quercetin′s polyphenol structure like the other flavonoids, makes it very sensitive to changes in the surroundings, which can modulate quercetin properties and consequently its stability. In this since, a very recently, spectrofluorimetric method based on formation of stable supramolecular complexes between quercetin and micelles of surfactants (cetyl trimethylammonium bromide, sodium dodecyl sulfate and triton X-100) was proposed [3].

In this work, micelles of surfactant (CTAB) was used to improve quercetin UV-VIS spectrophotometric quantification. Thus, the aim of this work is development of a new UV-VIS spectrophotometric method for quercetin determination based on interactions of quercetin with cationic surfactant cetyl trimethylammonium bromide, and, in doing so its applicability.

EXPERIMENTAL

Quercetin×2 H_2O, methanol and ascorbic acid were obtained from Merck (Darmstadt, Germany) and were used without any further purification. Quercetin + C capsules were available from Twin Laboratories Inc., Ronkonkoma, New York, USA.

Spectrophotometric measurements were performed on UV-VIS Spectrophotometer Beckman DU-650 (Fullerton, USA), using a 1 cm quartz cuvette. For estimation of calibration curve for Q quantifications, absorption spectra of solutions with different Q concentrations in 3% methanolic solution (2.0×10^{-6} –8.0×10^{-5} mol dm^{-3}) and the constant CTAB concentration (2.0×10^{-3} mol dm^{-3}) were recorded in the range of wavelengths 220 – 500 nm.

For Q determination in a pharmaceutical preparation, the sample solution was prepared to dissolve an amount equivalent to the average weight of two capsules (containing 500 mg of quercetin and 1400 mg of vitamin C) in 200 cm^3 of methanol, shaking for 15 min in ultrasonic bath and filtered through filter paper Whattman No 1. The obtained solution is diluted with methanol/water and CTAB solution to the concentration of 5 μg cm^{-3}.

RESULTS ANS DISCUSSION

In the wavelength range 220 – 500 nm, 3% methanolic solution of Q shows two major absorption bands in the UV – VIS region: band I located in the wavelength range of 300 – 400 nm (ie. at 364 nm) is related to the cinnamoyl system, and band II located in the wavelength range of 240–300 nm (i.e. at 252 nm) is supposed to be associated with the light absorption of the benzoyl moiety (curve a, Fig. 1.).

UV-VIS quercetin absorption spectra showed hypochromic, hyperchromic and bathochromic effects upon addition of CTAB (Fig. 1, curves b-e). The absorbance of the band I increases (hyperchromic effect), while the band II is affected in two manners: there is decrease (hypochromic effect) and increase (hyperchromic effect) in the absorbance, for CTAB concentration smaller and higher than critical micelle concentration (CMC), respectively. Besides to that, there is a slight shift of bands towards higher wavelength region (bathochromic effect); presence of CTAB in concentration very close to CMC and higher then CMC cause a bathochromic shift of about 10-15 nm from the original band I in the absence of CTAB (curves c and d, Fig. 1.). Namely, formed CTAB spherical micelles (after CMC) shift band I from 364 nm to 379 nm. On the other hand, for CTAB concentration higher than 2 mmol dm^{-3}, the band I position is not changed (curve e, Fig. 1). It is obvious that the best conditions for Q quantification relate to the λ = 379 nm and c_{CTAB} = 2 mmol dm^{-3}. In addition, in the presence of CTAB micelles, no change of quercetin absorbance in the time at λ = 379 nm. Thus, the use of CTAB micelles provides enough stability to Q during its spectrophotometric quantification time.

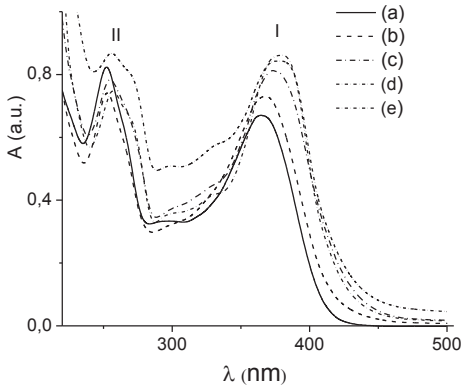

Figure 1. UV-VIS absorption spectra of 3% methanolic solution of Q (0.04 mmol dm^{-3}) alone (a) and in presence of different CTAB concentrations (in mmol dm^{-3}): 0.5 (b), 0.8 (c), 2.0 (d) and 4.0 (e)

It is evident that interaction between Q and CTAB promotes absorption of the former when the CTAB concentration is higher than the respective CTAB critical micellar concentration (about 1 mmol dm^{-3}). Thus, for quercetin quantification, the absorption spectra of solutions with constant CTAB concentration (2 mmol dm^{-3}) and different Q concentrations (2.0×10^{-6} -8.0×10^{-5} mol dm^{-3}) at λ = 378 nm, were recorded.

The absorbance of these micellar systems at selected wavelength varies linearly with the Q concentration. Method validation was done in accordance with ICH guidelines (table 1). Under selected experimental conditions, Beer's law was valid within a concentration range of about two orders of magnitude (2×10^{-6} – 8×10^{-5} mol dm^{-3}). The calibration curve, has the regression equation, A = 21133 c – 0.025 (r = 0.9991). The sensitivity of the proposed method was 2.1×10^4 dm^2 mol^{-1} (the molar absorptivity) as well as 1×10^{-6} mol dm^{-3} (the limit of detection).

The stability of the supramolecular assembles between Q and CTAB micellar system allows to apply the method described above for the Q determination in pharmaceutical preparation (*Quercetin + Vitamin C capsules*). Although the presence of ascorbic acid may cause problem in measurements, quercetin has been determined successfully (Table 1).

Table 1. Precision and recovery of quercetin in capsules

Quercetin +Vitamin C capsules	Found Q-dihydrate (mg)	Recovery (%)	SD (%)	CV (%)
Declared 250 mg of per capsule	246.3	98.5	0.23	0.093

CONCLUSION

Our results demonstrate the feasibility of the proposed spectrophotometric method based on formation supramolecular CTAB–Q assembles for quercetin quantification in presence of ascorbic acid. The developed method shows good analytical features; the results are accurate and precise, and there are advantages in terms of short time required for each assay.

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