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Direct spectrophotometric determination of quercetin in the presence of ascorbic acid

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Abstract

The current research provides a simplified sample preparation procedure for the accurate estimation of quercetin in pure and in the pharmaceutical dosage form. Direct spectrophotometric method for the determination of quercetin in the presence of ascorbic acid was established. The influences of medium, wavelength, pH, temperature and the ionic strengths on quercetin determination were investigated. The best conditions for calibration curve are: 50% ethanol, λ = 370 nm, pH = 4.2, T = 34 °C and $I = 7.5 \times 10^{-5}$ M. Beer's law is obeyed in the concentration range 1.0–12.0 μg ml^{-1} for quercetin. The corresponding detection limit is 0.76 μg ml^{-1}. The proposed method was verified by analyzing *Quercetin + C* capsules, Twinlab®.

Keywords: Quercetin; Vitamin C; Spectrophotometric method

1. Introduction

Among all the flavonoids, which display a significant array of pharmacological activity, quercetin (2-(3,4-dixydroxyphenyl)-3,5,7-trixydroxy-4H-1-benzopyran-4-one) is most commonly presented in food such as onions, tea, apples and red wine [1,2]. Quercetin is important dietary constituent because it is most widespread and consequently, the most studied flavonoid. A lot of articles are dealing with beneficial pharmacological properties of quercetin in the field of allergy, vascular, inflammation, virology and carcinogenesis [3–6]. As all flavonoids, quercetin exhibits potent antioxidant activity, due to free radical scavenging [7]. A numerous pharmaceutical preparations containing quercetin (mostly with vitamin C or with other flavonoids) are available on the market. As a result, simplified analytical technique is required for the quercetin determination in food and pharmaceutical dosage forms.

Various methods have been described for the determination of quercetin mostly from plant materials: high-performance liquid chromatography [8–10], capillary electrophoresis [11] and derivative spectrophotometry [12]. Quercetin was also determinated spectrophotometrically via coloring complexing reaction with many different inorganic reagents added [13,14].

The aim of this work is to simplify already established spectroscopy methods for quercetin determination. The detection limit of quercetin can be significantly improved (without any reagents added) by simply selection of optimum parameters such as solvent, pH, buffer, wavelength, the ionic strength and temperature [15]. Since flavonoids and vitamin C can often be found together in pharmaceutical preparations, the proposed method must be suitable for quercetin determination in the presence of vitamin C.

2. Experimental

2.1. Apparatus

Spectrophotometric measurements were performed on Spectrophotometer Beckman DU-650 (Fullerton, USA), using a 1-cm quartz cuvette. pH-meter (pHM-82 Radiometer Copenhagen), accuracy of ±0.001 pH, equipped with the combined electrode (No. CW. 733 Series No.35162, Russel) was used. The temperature was controlled within ±0.2 K by circulating water thermostat (Series U, MLW Freital, Ger-

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many). Ultrasonic bath is supplied by L.U.5, 7 Fungilab, S.A., Spain.

2.2. Reagents

Quercetin × $2H_2O$, ascorbic acid, ethanol and $NaNO_3$ were obtained from Merck (Darmstadt, Germany). Succinate buffer, suitable for ethanol–water mixtures, was prepared according to prescribed procedure [16]. All reagents were of PA grade and were used without any further purification. *Quercetin + C* capsules were available from Twin Laboratories Inc., Ronkonkoma, New York, USA.

2.3. Standard solutions

The standard stock solution of quercetin ($c = 5 \times 10^{-4}$ M) was prepared by dissolving 42.3 mg of quercetin in 250 ml absolute ethanol. To prepare standard curves, appropriate volumes of stock solution were transferred and diluted to final concentrations of 0.5, 1, 2, 3, 5, 7, 9,10, 12 and 15 μg ml^{-1}. All standard solutions were prepared in 20-ml volumetric flasks, by mixing appropriate volumes of the quercetin standard stock solution (V_1), absolute ethanol (V_2), H_2O (V_3) and the succinate buffer (V_4). To obtain final solutions of the required molar concentrations in 50% ethanol, it is necessary that $V_1 + V_2 = 10$ cm^3 and $V_3 + V_4 = 10$ cm^3. As volumes are not strictly additive, the volumetric flasks were filled to the mark with 50% ethanol.

2.4. Sample solution

For the quercetin determination in *Quercetin + C* capsules, 20 capsules were weighted and average value was calculated. The sample solution was prepared dissolving an amount equivalent to the average weight of two capsules (containing 500 mg of quercetin and 1400 mg of vitamin C) in 250 ml of ethanol, shaking for 15 min in ultrasonic bath and filtered through filter paper Whattman No 1. The obtained solution is diluted with ethanol/water and buffer to the concentration of 5 μg ml^{-1} in 50% ethanol. The procedure was repeated five times.

3. Results and discussion

3.1. Influence of solvent, pH, ionic strengths and temperature

The optimum medium for direct quercetin determination related to 50% ethanol. Solvent with this percentage of ethanol enables the best compromise between quercetin solubility (quercetin is hardly soluble in pure water) and sufficient absorbance (water favorites quercetin dissociation and consequently its absorbance).

The influence of pH and the ionic strengths on absorption spectra of quercetin, at the constant value of temperature ($T = 25$ °C) are investigated and presented in the Fig. 1. From the Fig. 1, it is obvious that the best conditions (the higher absorbance) relate to the pH range from 4.2 to 6.9 (curves 1–5), the value of ionic strength $I = 7.5 \times 10^{-5}$ M and wavelength $\lambda = 370$ nm.

The influence of temperature on the maximum absorption of quercetin solution at selected ionic strength is investigated and results are presented in the Fig. 2. The obtained curve

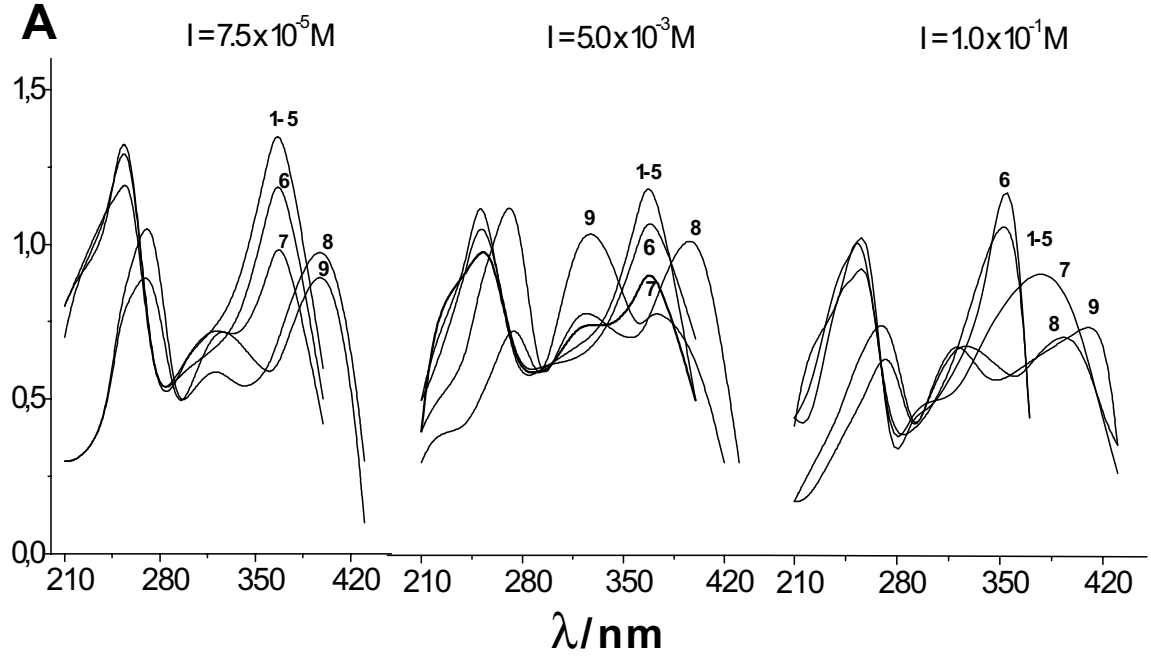

Fig. 1. The dependence of absorption spectra of quercetin on pH obtained for three different values of ionic strength. $c_{Quercetin} = 15$ μg ml^{-1}, curves 1–5: pH from 4.2 to 6.9; curve 6: pH = 7.2; curve 7: pH = 8.2; curve 8: pH = 9.2 and curve 9: pH = 10.0. Blank for all measurements is 50% ethanol in the same pH, the ionic strength and temperature as in the quercetin solution.

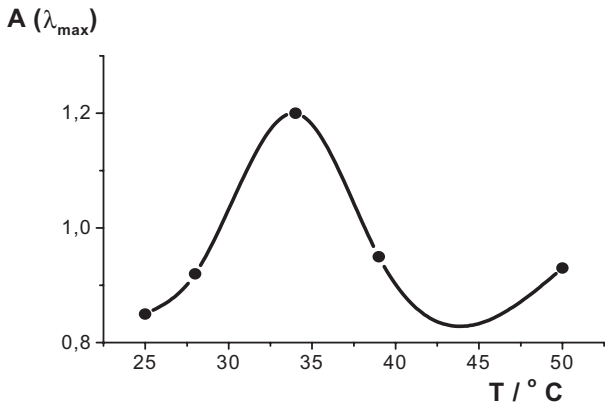

Fig. 2. The dependence of temperature on the absorption maximum of the quercetin solution. $c_{Quercetin}$ = 10 μg ml^{-1}, pH = 4.2, λ = 370 nm and I = 7.5 × 10^{-5} M.

shows the maximum value of absorbance for the temperature T = 34 °C.

3.2. Influence of ascorbic acid

After fixing all investigated parameters, the influence of ascorbic acid on absorption spectra of quercetin is explored. The evident change of absorbance of quercetin in time is a result of potent oxidation of ascorbic acid (Fig. 3).

3.3. Analytical appraisal

Based on previously described examination, the best conditions for direct spectrophotometric quercetin determination correspond to 50% ethanol as solvent, λ = 370 nm, pH = 4.2, the value of ionic strength I = 7.5 × 10^{-5} M and T = 34 °C. Beer's law was valid within a concentration range from 1 to 12 μg ml^{-1}. The calibration curve, obtained under these conditions, has the regression equation, A = 0.1307c + 0.002, with a good correlation coefficient (r = 0.9991). The detection limit (signal-to-noise ratio = 3) was 0.76 μg ml^{-1}. All measurements have to be performed after 4 min, since ascorbic acid is easily oxidabling and influences on the absorption maximum of quercetin (Fig. 3).

3.4. Method precision

The method was tested for precision, sensitivity and reproducibility. Precision of the method was checked at three different concentrations by performing seven replicate determinations within Beer's law limits. Precision of method is evident from the maximum standard deviations (S.D.) lying between 0.03 and 0.09 for three checked concentrations. The r.s.d. values, which are less, than 3% for three different levels studied indicate the high reproducibility of the method. From the values of r.s.d., the concentration of 5 μg ml^{-1} was selected for the further investigation.

3.5. Application

The applicability of the method for the assay of the sample was tested with *quercetin + C* capsules (Table 1). The low value of S.D., r.s.d. less than 3% and recovery lying in stated range (Ph EUR 97), indicate good application of method.

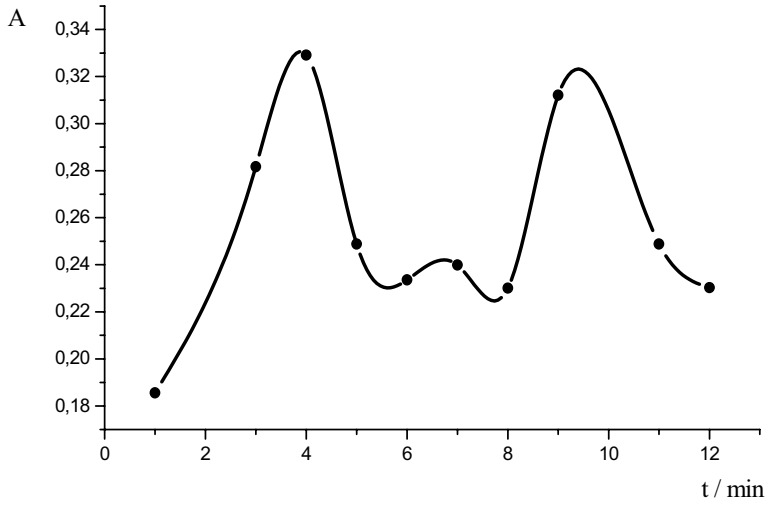

Fig. 3. The influence of ascorbic acid on absorption spectra of quercetin. Dependence of absorbance of quercetin on time of record. $c_{Quercetin}$ = 3 μg ml^{-1}, c_{VitC} = 8.4 μg ml^{-1}, pH = 4.2, λ = 370 nm, I = 7.5 × 10^{-5} M and T = 34 °C.

Table 1
Precision and recovery of quercetin in bulk drug and capsules

Sample	Concentration (μg ml^{-1})	Found ± S.D. (μg ml^{-1})	r.s.d. (%)	Recovery (%)
Quercetin bulk drug	3	2.96 ± 0.06	2.03	98.67
	5	4.98 ± 0.03	0.6	99.6
Quercetin + C capsules	7	6.87 ± 0.09	1.31	98.14
	5	4.82 ± 0.13	2.7	96.4

n = 7 runs.

Compared to aforementioned spectrophotometric methods for quercetin determination, the proposed method provides lower detection limit without any reagents being added [14]. The developed method, as simple and economical, can be recommended for the routine analysis of quercetin in the presence of vitamin C.

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