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Three-dimensional strategies in the quantitative resolution of kinetic UV absorbance measurements for monitoring the oxidation of quercetin by oxidant agents and analyzing dietary supplement product

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Abstract

Three-dimensional strategies involving the application of parallel factor analysis (PARAFAC) to the kinetic UV absorbance measurements were elaborated to monitor the oxidation of quercetin with oxidant agents ($K_2Cr_2O_7$ and KIO_3) and to quantify analyte in a dietary supplement product. Loadings (spectral, kinetic and concentration profiles) were obtained by the PARAFAC deconvolution. Spectral identification, kinetics and quantification of the relevant analyte in the presence of interferent(s) were performed. The elaborated chemometric strategies were carefully validated to demonstrate the capability of the method. Assay results of the PARAFAC strategies were statistically compared to that of the newly developed UPLC method.

Keywords: Chemometric strategies, Dietary supplement product, Kinetic UV absorbance, PARAFAC, Quercetin, UPLC

1. Introduction

Nowadays, fruits and vegetables, especially plants containing functional compounds have increasingly been interest in terms of preventive human health. Polyphenolic substances, especially flavonoids such as quercetin, procyanidin, chlorogenic acid, epicatechin and vitamin C are consumed daily by humans, either naturally or through food supplements, and are of great interest because of their high bioactivity. Moreover, they have numerous biological and pharmacological effects, such as antioxidant, anticancer, antiallergic, anti-inflammatory, antiviral and cardioprotective activities [1–6].

Quercetin is one of the most abundant flavonoids and found naturally in fruit peels, leafy vegetables, strawberries, onions, cranberries, blueberries, black tea, red wine and various fruit juices [7–10]. Its IUPAC name is 3,3′,4′,5,7-pentahydroxyflavone and its chemical structure has a carbonyl group in position 4, a double bond between carbons 2 and 3, and five hydroxyl groups in positions 3,5,7,3′,4. In particular, the three hydroxyl groups in positions 3, 3′, and 4′, make it one of the most potent of the congeners [10]. In previous reports, the average human consumes approximately 16–25 mg of quercetin per day from food sources [11]. Quercetin is also available in powder and tablet form as a nutritional supplement [12]. Typical daily dosages vary from 500 to 1000 mg [6]. The Food and Drug Administration (FDA) of the United States considers quercetin to be a relatively safe substance [13]. Consequently, intake of a quercetin-rich diet is supported and is positively correlated with health promotion [14,15].

Various studies have been reported in the literature on the antioxidant activity [16–18] and degradation [17–19] of quercetin using enzymatic or

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chemical agents with instrumentation techniques involving spectrophotometric, electrochemical or chromatographic methods.

Some of polyphenolic compounds, such as quercetin, have been found to be activate non-enzimic and enzimic browning of foods [18]. In most cases, the reactions take place in oxidative steps and subsequent, lead to the change in the chemical and biologic characteristics of related molecules. In general, the oxidation of flavonoid degradation in plant tissue have been made with two different enzymes, polyphenol oxidase (PPO), and peroxidase (POD). Oxidations with chemical agents such as potassium ferricyanide, H_2O_2 and sodium periodate have been studied to compare the flavon oxidation with enzimatic agents and to perform model oxidations [17–19]. In this context, new functional oxidative agents are to find for the degradation of quercetin using model oxidations.

Quercetin has been widely used as an active compound in dietary supplement and pharmaceutical preparations. In this regard, quality control and routine analysis of quercetin is indispensable before and after industrial preparation.

In previously published articles, quercetin in fresh fruits and vegetables was analyzed using various analytical techniques including gas chromatography (GC–MS) [20], UV–Vis spectrophotometry [21], liquid chromatography (HPLC) [13], capillary electrophoresis [22], solid phase extraction [23], spectrofluorometry [24] and electrochemical methods [25]. It was determined in commercial food products (or dietary supplements) using HPLC [13], spectrophotometry [26], spectrofluorometry [27] and reverse-phase HPLC methods using C_{18} column [28]. In addition, the amount of quercetin alone in biological liquids, plant samples and food samples was quantified by different analytical methodologies involving UV–Vis spectrophotometry [21], fluorometric detection [10], solid phase extraction [23] and electrochemical methods [25]. Simultaneous quantitative analysis of quercetin in complex mixtures with other active compounds have been reported in a few publications. They were estimated in human plasma and human urine using HPLC [28,29], in plants using capillary electrophoresis with electrochemical detection [22], in red wine using UV-VIS-NIR spectroscopy [30] and in pharmaceutical tablets using UV-spectrophotometry [31].

Usually, HPLC and spectrophotometry have been used to monitor the degradation of analyte in chemical reaction and analyze complex mixture. Especially, the application of spectrophotometry is preferable instead of HPLC to solve the mentioned issues due to simplicity and low cost. For instance, HPLC method has a large reagent consumption and long analysis time. As for UV–Vis spectrophotometry, it may not always provide desirable results for the assay of degradation and quantification due to overlapping signals of components or interference of main compound with sample matrix. The main objective in analytical studies is to eliminate the disadvantages of traditional methods (HPLC and spectrophotometry) and to develop a cheaper and simpler method for the degradation and determination of active compounds. This would be possible by three-way (or N-way) analysis of kinetic UV–Vis spectrophotometric measurement to monitor kinetic degradation of analyte and its quantification in complex samples.

In the present work, two novel chemometric strategies involving the application of a three-way analysis model to two different three-way arrays of kinetic UV spectroscopic measurements were proposed to monitor the kinetics of oxidative reactions of quercetin by oxidant agents, $K_2Cr_2O_7$ and KIO_3, and to quantify the content of quercetin in commercial dietary supplement product without using any preliminary separation or chemical pretreatment. Three loadings, also named as spectral profile, kinetic profile and concentration profile were obtained by the decomposition of the kinetic UV spectroscopic datasets using PARAFAC model. Then, the identification of chemical components in spectral profile, the observation of chemical species in kinetic reaction medium and the determination of quercetin from concentration profile were carried out without using any preliminary separation or chemical pretreatment. For a comparison of the assay results obtained with the proposed chemometric strategies, a reverse-phase ultra-performance liquid chromatography with photodiode array detection (RP-UPLC-PDA) was developed for the assay of quercetin in the same samples. The precision, accuracy and selectivity of the proposed chemometric strategies and RP-UPLC-PDA were verified by the analysis of test samples and spiked samples, and they were provided satisfactory results. The improved and validated methods were subjected to the quality control and routine analysis of the commercial dietary supplement product containing quercetin. This study showed that three-dimensional chemometric strategies and chromatographic approach were precise, accurate and selective assay results in terms of the method applicability. All the methods were statistically compared with each other using one-way ANOVA test.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used are of analytical grade. Quercetin was supplied from Sigma (Steinheim, Germany). Analytical grade methanol (J.T. Baker, Netherland), acetonitrile (Sigma–Aldrich, Steinheim, Germany), phosphoric acid (Sigma Aldrich, USA), sodium hydroxide (Riedel-de-Haen, Seelze, Germany), Sodium dihydrogen phosphate dihydrate (Sigma–Aldrich, Steinheim, Germany), potassium iodate (Kimetsan, Ankara, Turkey), potassium dichromate (Merck, Darmstadt, Germany) and TEA (trimethylamine) (Sigma–Aldrich, Belgium) were used. The commercial dietary supplement product, named as Calcium Duo Alergo, was supplied from Chopin's POLISH DRUG 10 Wadowice, Polish. The content of this commercial product consist of active compound (quercetin), acidity regulator (citric acid, sodium bicarbonate, calcium carbonate), bulking agent (sorbitol, corn starch), anti-caking agent (polyvinylpyrrolidone) and sweeteners (acesulfame K, aspartame). Solutions of reagents $K_2Cr_2O_7$ and KIO_3 used in spectrophotometric and UPLC studies were prepared in ultrapure water obtained from Milli-Q Gradient A10 Millipore Purification System (Merck Millipore, USA). The same ultrapure water was used for the preparation of phosphate buffer system used in the mobile phase. Prior to UPLC analysis, filtration of all analysis solutions was performed using a PVDF (Polyvinylidene Difluoride) syringe filter (Isolab, Wertheim, Germany) with a 0.22 μm pore size.

2.2. Preparation of assay solutions

A stock solution of quercetin was prepared by dissolving 10 mg of pure substance in methanol in a 100-mL calibrated flask and stored at room temperature. Standard solutions (calibration) at five different concentrations (4, 8, 12, 16 and 20 μg/mL) were prepared from the stock solution. The inter-day and intra-day samples in three levels (5, 10 and 20 μg/mL) were prepared in the same way to get method validity. The spiked samples were prepared by adding the standard solution of quercetin at four concentration levels (0, 4, 8 and 12 μg/mL) to 0.6 mL of the solution of the commercial dietary supplement product in 10-mL calibrated flask. Each experiment was repeated three times for the preparation of calibration, validation and commercial samples. Oxidant reagents were prepared by dissolving 0.125 g potassium dichromate or potassium iodate in ultrapure water in a 25-mL calibrated flasks and stored at room temperature.

2.3. Instrument analysis and software used

A Shimadzu double-beam UV-VIS spectrophotometer (Shimadzu UV-2550, Japan) with 0.2 nm slit width connected to a desktop computer was used to record kinetic UV spectra of samples. For the oxidation reaction of quercetin in the analyzed samples by $K_2Cr_2O_7$ and KIO_3 whose strategy I and strategy II were categorized into two different procedures, the kinetic UV spectra were recorded. The kinetic UV spectroscopic data recorded were transferred to the Microsoft Excel program (Microsoft, USA) and then, they were processed by three-way analysis algorithm. Multiway data processing was made by using the written special m-file together with the N-way Toolbox [32] in Matlab software (Mathworks Inc., USA). All graphs and figures were plotted by means of Matlab software.

The RP-UPLC-PDA study was performed using water H-class UPLC instrument (Acquity Waters) and Acquity UPLC BEH Phenyl analytical column (1.7 μm 2.1 × 100 mm) (Waters, USA). A mixture of methanol and 0.1 M phosphate buffer (pH 2.15) solution with 0.4 mL TEA/L (55:45, v/v) was used as a mobile phase system with a flow rate of 0.20 mL/min. PDA detection was made at the wavelength of 245 nm. Empower 2 software was used to control the chromatographic system and to acquire chromatographic data. Regression analysis for the calibration curves was accomplished by Microsoft Excel program. A magnetic stirrer (Heidolph, Germany) was used to carry out the dissolution process of commercial effervescent tablets.

2.4. Determination of quercetin in commercial effervescent tablets

The analysis of commercial dietary supplement product consisting of effervescent tablets was performed by using the following procedure. Five commercial effervescent tablets were weighted and powdered in a mortar. An adequate tablet amount of this powder was dissolved in methanol in a100-mL calibrated flask. The content of the flash was stirred for 4 h at 50 °C temperature with magnetic stirrer and then filtered by using PVDF syringe filter (Isolab, Wertheim, Germany) with 0.22 μm pore size into calibrated flask. The appropriate volume of filtrate was added to a 10 mL calibrated flask, and one of these $K_2Cr_2O_7$ and KIO_3 (according to strategy I or

strategy II) was put in the flask to take place a reaction between the analyte and oxidant agent. Then the kinetic spectrum of the sample was plotted.

2.5. Statistical analysis

Statistical analysis was performed using one-way ANOVA (analysis of variance) test to compare the assay results involving the averages of the determinations of quercetin in dietary supplement product samples using chemometric strategies and UPLC method. One-way ANOVA test was performed with Microsoft Excel software in Office 2019.

3. Results and discussion

UV–Vis spectrophotometry is one of the most widely used methods to investigate or elucidate the kinetics and mechanism of chemical reactions due to some advantages such as simplicity of the method, low cost, simple sample preparation and measurement. However, the main problem of UV–Vis kinetic spectrophotometric studies is the overlap or interference of spectral bands of chemical species in complex reactions or multicomponent mixtures. To achieve accurate and reliable results for each component in the reaction medium, overlapping bands of chemical species in a reaction or in the presence of unknown interferences can be resolved with appropriate multiway data analysis approaches. In this context, the application of three-dimensional spectrophotometric analysis requires a matrix data (e.g. wavelength × time). Therefore, in our case, the kinetic UV spectra were recorded as a function of wavelength (nm) and time (min) to get a two-way data matrix. For this purpose, UV–Vis spectrophotometric data sets were created based on the kinetic reactions of quercetin with oxidant agents in the presence of sample matrix. Individual contributions of components in the kinetic reaction medium were obtained from the spectral, kinetic and relative concentration profiles using three-way analysis of spectral datasets. Two different chemometric strategies and their applications in kinetic UV data arrays were described below.

3.1. Kinetic UV absorbance measurement and data acquisition

In preliminary experiments, some oxidizing agents were tested to determine the desired oxidation of quercetin in methanolic solution. From these experiments, two agents, $K_2Cr_2O_7$ and KIO_3, were found to be suitable for monitoring the kinetic oxidation of quercetin and measuring its content in commercial dietary supplement product. Three-dimensional kinetic UV spectra for quercetin oxidation in samples with $K_2Cr_2O_7$ were recorded between 225.0 and 545.2 nm (0.2 nm increments) and 0.0–20.0 min (5 min increments) for example, as shown in Fig. 1a and b for the calibration and dietary supplement samples. In this study, the analyzed samples consisted of calibration set, test samples, spiked samples and dietary supplement samples. In the next step, second-order kinetic UV absorbance matrices were collected and arranged as a cubic data array with dimensions, wavelength × time × sample (1626 × 5 × 31). Three dimensions (wavelength, time and sample), expressed by 1626 × 5 × 31, for the cubic data array (or three-way array) represent the absorbance at 1626 wavelength points, the change in time (between 0.0 and 20.0 min with 5 increments), and the number of samples (from 1 to 31).

In the similar manner, three-dimensional kinetic UV data matrices for the oxidation of the quercetin in the above mentioned samples using KIO_3 were obtained as a function of the wavelength in the region of 234.0–460.0 nm (0.2 nm increments) and time in the range of 0.0–20.0 min (5 min increments). As an example, for the reaction of the quercetin with KIO_3, three-dimensional spectra for the calibration set and commercial dietary supplement samples were illustrated in Fig. 2a and b. From these kinetic UV data matrices, a three-way data array with dimensions, wavelength × time × sample (1131 × 5 × 31) was created.

Herein, newly developed two procedures based on three-way resolutions of kinetic UV data measurements obtained by the oxidation of quercetin by $K_2Cr_2O_7$ and KIO_3 were categorized as strategy I and strategy II, respectively, to visualize two oxidation procedures.

In the practical implementation of the strategy I, the kinetic oxidation of quercetin by $K_2Cr_2O_7$ was performed for each sample of the calibration set (4, 8, 12, 16 and 20 μg/mL). In this assay, 0.4 mL of quercetin at 10 mg/100 mL was transferred to a 10-mL calibrated flask, followed by 155 μL of 0.5% $K_2Cr_2O_7$ solution, which is corresponded to 2.66×10^{-3} mmol. The volume was made up to the mark with methanol. After shaking the content of the solution, it was placed in a quartz cuvette (Hellma, Germany) and kinetic UV spectra were recorded in the range of 225.0–545.2 nm for 20 min in five replicates (5 min increments) (see Fig. 1a).

For the assay of the strategy II, the kinetic oxidation reaction of quercetin was established by using KIO_3 instead of $K_2Cr_2O_7$ agent. As in the implementation of strategy I, the kinetic reaction in

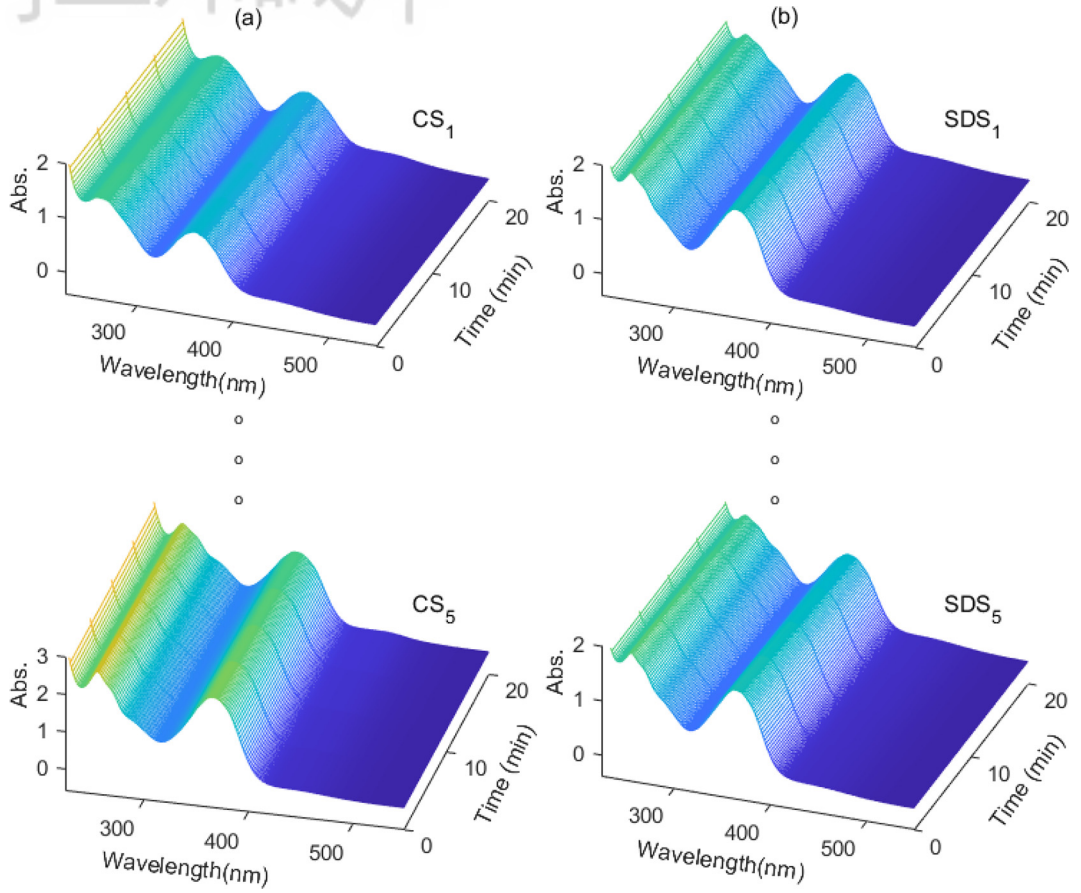

Fig. 1. Three dimensional kinetic UV spectra of the oxidation of quercetin with $K_2Cr_2O_7$ in (a) calibration samples (from CS_1 to CS_5) and (b) samples of dietary supplement (from SDS_1 to SDS_5). CS_1-CS_5 denotes the calibration samples and SDS_1-SDS_5 denotes the samples of dietary supplement.

strategy II was performed by the oxidation of quercetin in calibration, validation, and commercial dietary supplement samples using KIO_3. In this assay, for the kinetic oxidation reaction of the first sample (4 μg/mL quercetin) of the calibration set, 0.4 mL of quercetin, which is the concentration of 10 mg/100 mL, was added to a 10-mL calibrated flask and in the next step, 57 μL of 0.5% KIO_3 (1.33×10^{-3} mmol) solution was filled into it to start the reaction. The volume of the calibrated flask was made up to the mark with methanol and the contents of the flask were shaken by a manual manipulation. The reaction mixture obtained from the above procedure was filled in a quartz cuvette with 1 cm path-length. Its kinetic UV spectra were plotted as a function of wavelength and time in the range of 234.0–460.0 nm (0.2 nm increments) and 0.0–20 min (5 min increments) (see Fig. 2a). Then, a two-way data matrix with two-dimensions, wavelength × time (1131 × 5) was obtained from the kinetic UV spectra of the first sample of the calibration set. In the strategy II, the above procedure for each sample in calibration, validation and real dietary supplement product samples was repeated and then, a three-way array with dimensions, wavelength × time × sample (1131 × 5 × 31) was obtained.

3.2. PARAFAC analysis of kinetic UV data arrays

As previously described, two different three-way arrays of kinetic UV absorbance measurements (1626 × 5 × 31 and 1131 × 5 × 31) were obtained during the oxidation reactions of quercetin in the analyzed samples by $K_2Cr_2O_7$ and KIO_3, respectively. In preliminary applications of strategy I and II, the PARAFAC algorithm using or not using constraints, and with a different number of components (or factors) was tested for the above three-way arrays to fit the model to data. In these procedures, three, four, five and six factor models were applied to the three-way tensors. Four factor PARAFAC model was found to be suitable to describe the observed kinetic oxidation reactions of quercetin with $K_2Cr_2O_7$ and KIO_3. However, this four factor model generated partially negative shapes in three profiles obtained from the decomposed three-way

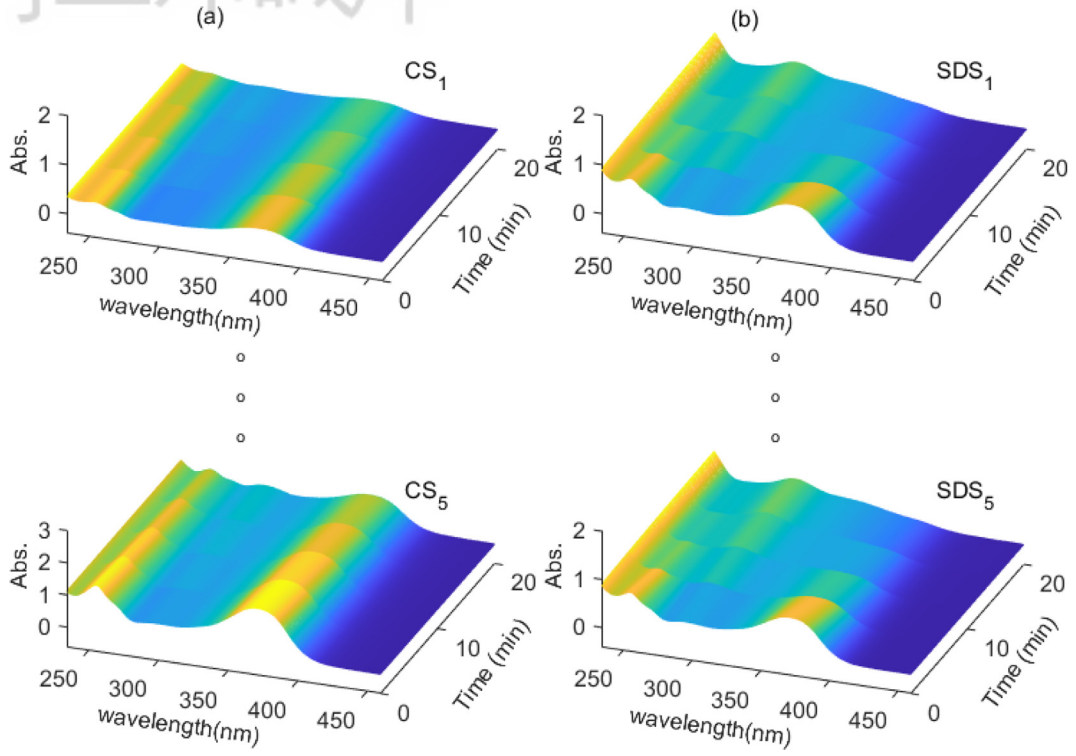

Fig. 2. Three dimensional plots of kinetic UV spectra of the oxidation of quercetin with KIO_3 in (a) calibration samples (from CS_1 to CS_5) and (b) samples of dietary supplement (from SDS_1 to SDS_5). CS_1-CS_5 denotes the calibration samples and SDS_1-SDS_5 denotes the samples of dietary supplement.

arrays (1626 × 5 × 31 and 1131 × 5 × 31). Therefore, the application of four factor PARAFAC model, the use of non-negative constrains in three modes was improved the fit of data and then, all three resolved profiles were found to be logical in terms of the monitor of kinetic oxidation reactions of the analyte with $K_2Cr_2O_7$ and KIO_3.

In the chemometric strategy I, once the appropriate component number and constraint were correctly determined, three-way kinetic UV absorbance data obtained by the oxidation of quercetin in the 31 samples with $K_2Cr_2O_7$ were analyzed by the PARAFAC model to get the three profiles. Then, the normalized spectral, kinetic (time) and concentration profiles of the components in the kinetic reaction media were resolved. Fig. 3a (I), (II) and (III) show the individual curves of components (quercetin, $K_2Cr_2O_7$, oxidized form of quercetin and interferent) in three normalized profiles, which were corresponded to spectra, kinetic and concentration modes in the kinetic UV absorbance data array (1626 × 5 × 31). The UV spectra of the components in the reaction medium were identified from the normalized spectral profile as indicated in Fig. 3a (I).

The kinetic (or time) profile obtained indicates the spectral behavior of reactants and reaction product, and unknown component (or interferent) versus time in the kinetic oxidation reaction medium as illustrated in Fig. 3a (II). As seen in this figure, the kinetic curves of quercetin and $K_2Cr_2O_7$ decreased with reaction time, while the curve of the reaction product (or oxidized form of quercetin) increased with oxidation reaction time. From Fig. 3a (II), it was observed an increase in the kinetic curve of interferent (or unknown component). This seems a little oxidation of unknown component in commercial product with $K_2Cr_2O_7$. When this unknown component in strategy I was examined chromatographically, no peak was observed in the oxidation of pure quercetin with $K_2Cr_2O_7$, while a peak was observed in the oxidation of quercetin in the commercial product. For the oxidation of pure quercetin and quercetin in commercial sample by $K_2Cr_2O_7$, the obtained chromatograms were presented in Fig. 4a (I) and (II), respectively (the retention time for quercetin and product were 4.47 min and 2.30 min, respectively). Consequently, its curves were observed from the profiles in Fig. 3a (I), (II) and (III), due to the presence of unknown component in studied dietary supplement product. Similar situation was also observed for the oxidation of quercetin with KIO_3 as illustrated in Fig. 3b (II). To particularly

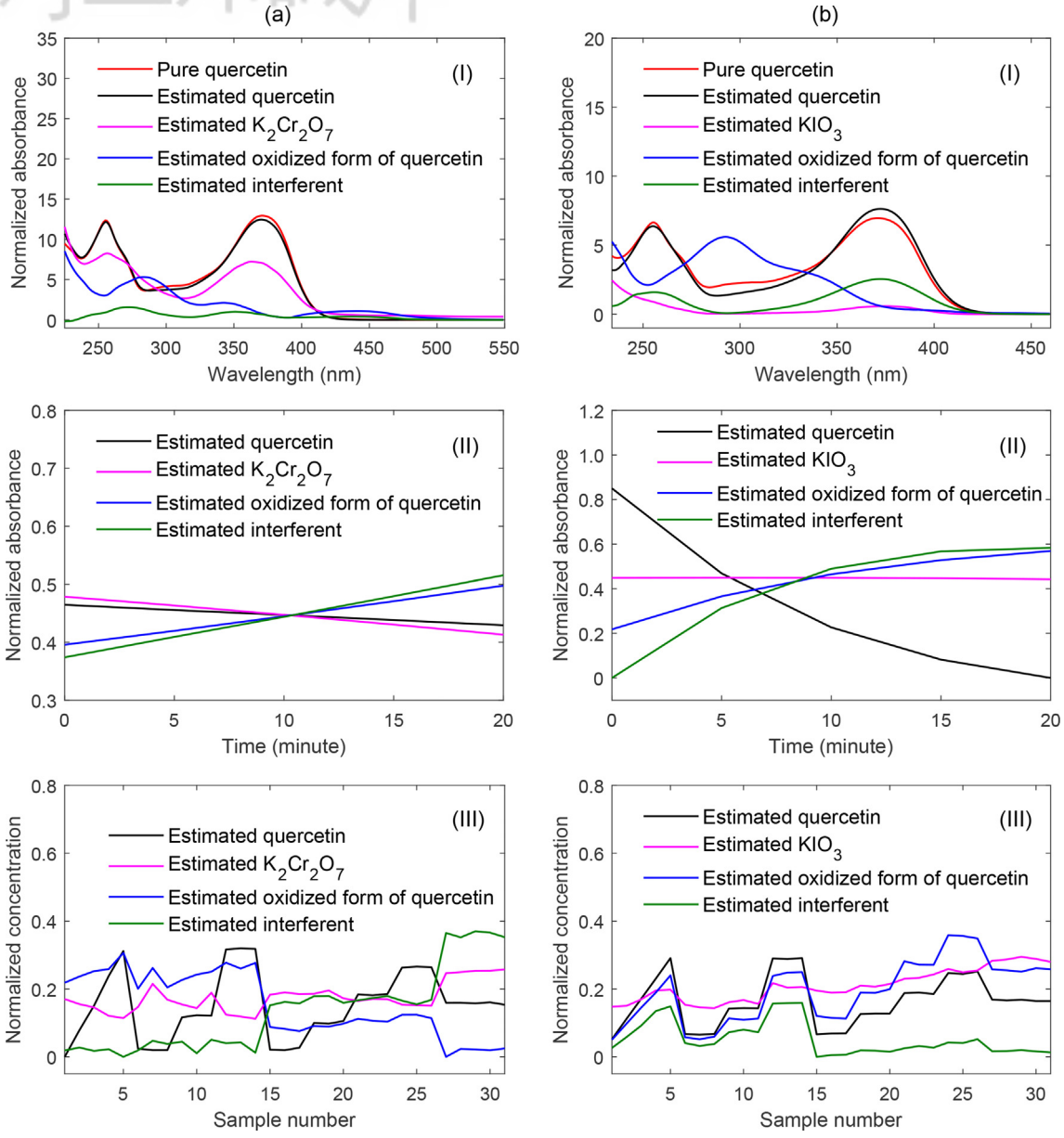

Fig. 3. I) Spectral profiles, II) kinetic profiles and III) relative concentration profiles of chemical species in the oxidation reaction of quercetin by two oxidant agents ($K_2Cr_2O_7$ (a) and KIO_3 (b), respectively) for the assay of calibration, validation and dietary supplement product using the PARAFAC model of kinetic UV absorbance measurements.

confirm kinetic behavior of the components in the reaction medium, the chromatograms were displayed in Fig. 4b (I) and (II), the oxidation of pure quercetin and quercetin in real sample by KIO_3, respectively (the retention time for quercetin and product were 5.78 min and 2.64 min, respectively).

The relative concentration profile for the related components in the kinetic oxidation reaction medium was indicated in Fig. 3a (III), obtained by applying four component PARAFAC model to the kinetic UV absorbance data tensor. From this relative concentration profile, the curve of quercetin was used for the quantitative estimation of the quercetin's content in validation and real commercial samples. For quantifying purpose, a calibration curve was obtained by the least squares regressions of the actual concentration on the relative concentrations obtained by the application of the PARAFAC model. The statistical results for the calibration curves obtained in the concentration range of 4–20 μg/mL of quercetin were listed in Table 1.

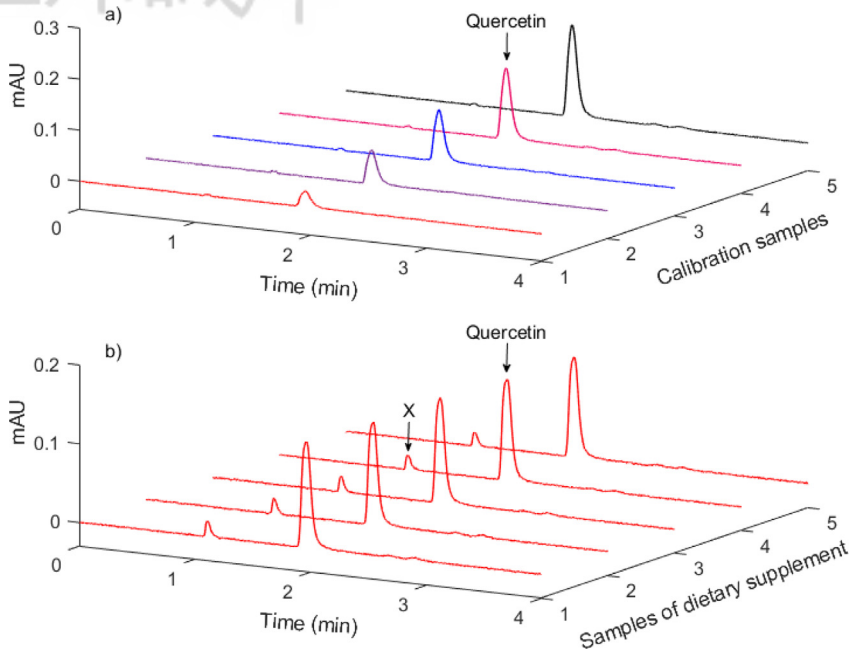

Fig. 4. UPLC chromatograms of a) calibration samples of quercetin in the concentration range of 4–20 μg/mL and b) samples of dietary supplement. In this chromatogram, X corresponds to the interferent or excipient in the analyzed sample.

Table 1. Calibration model results obtained by least squares regression analysis.

Parameter	Strategy I	Strategy II	UPLC
m	1.93×10^{-2}	1.86×10^{-2}	3.42×10^{-1}
n	-7.69×10^{-2}	-1.91×10^{-2}	-1.24×10^{-2}
r	0.9999	1.0000	1.0000
SE(m)	1.24×10^{-4}	8.87×10^{-5}	1.87×10^{-3}
SE(n)	1.65×10^{-3}	1.18×10^{-3}	2.48×10^{-2}
SE(r)	3.72×10^{-3}	2.75×10^{-3}	3.16×10^{-3}
LOD	0.26	0.19	0.22
LOQ	0.85	0.63	0.73

m: Slope of regression equation.
n: Intercept of regression equation.
r: Correlation coefficient.
SE (m): Standard error of slope.
SE (n): Standard error of intercept.
SE (r): Standard error of correlation coefficient.
LOD: Limit of detection (μg/mL).
LOQ: Limit of quantification (μg/mL).

3.3. RP-UPLC-PDA method

To compare the quantitation results of quercetin in the analyzed samples obtained by using two different PARAFAC models of kinetic spectrophotometric datasets, reverse-phase ultra-performance liquid chromatography with photodiode array detection (RP-UPLC-PDA) was developed to provide a convenient procedure for practical analysis of quercetin in effervescent tablets of commercial dietary product. In the RP-UPLC-PDA implementation, a suitable resolution and analysis was obtained an isocratic elution using a mobile phase consisting of methanol and 0.1 M phosphate buffer solution (pH 2.15) (55:45, v/v) containing 0.4 mL TEA/L. In the UPLC elution of analyte, the column system, Water C_{18} column (1.7 μm 2.1 × 100 mm) was used as a stationary phase. Quantification of quercetin in the related samples was made with PDA detection based on measuring the peak area. PDA detection of quercetin was carried out at 245 nm. The retention time of quercetin was 1.97 min with the run time of 4.0 min. The flow rate was 0.2 mL/min, and the injection volume was 1.0 μL.

The stock solution (10 mg/100 mL) was prepared in methanol. The calibration solution was prepared by dilution of the above stock solution with methanol to get a concentration range 4–20 μg/mL quercetin. Under the previously explained chromatographic conditions, injections were made and then chromatograms were obtained as illustrated in Fig. 5. Similar RP-UPLC-PDA procedures were applied to validation and commercial samples. In Fig. 5, the peak areas provided from RP-UPLC-PDA procedure were plotted against the concentrations in the calibration solutions to get the calibration curve. The statistical results for regression line of quercetin were presented in Table 1. This linear regression curve was used for the estimation of the content of quercetin in the studied samples. To view the effect of sample matrix on the analysis, standard addition technique was implemented. As in PARAFAC model with strategy I and strategy II, spiked samples were prepared and analyzed by RP-UPLC-

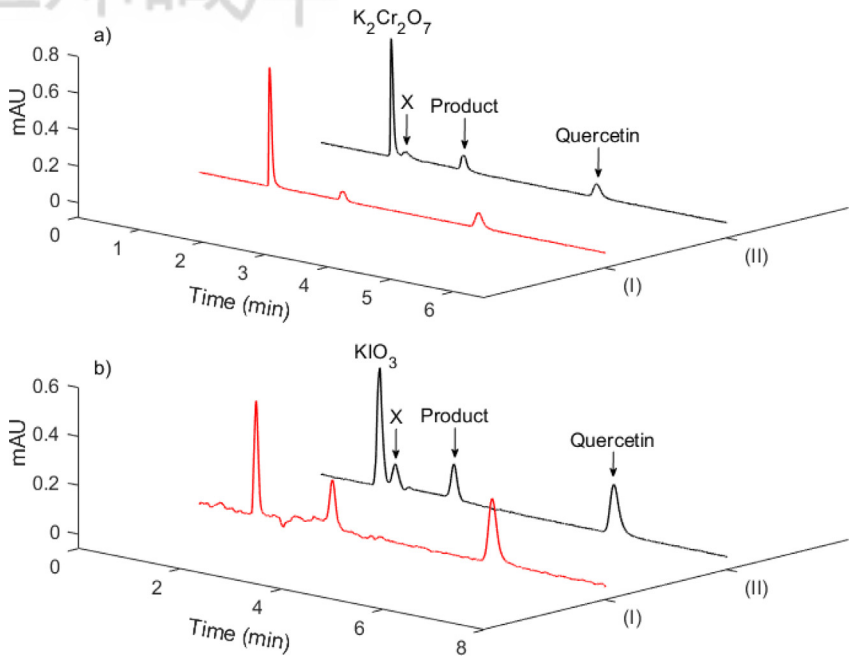

Fig. 5. UPLC chromatograms of a) $K_2Cr_2O_7$ and b) KIO_3 (I) pure quercetin, (II) commercial dietary supplement.

PDA. Their results were shown in Table 3. The assay showed that no interference of sample matrix was reported for the application of the UPLC technique to the analysis of the spiked samples.

For strategy I oxidation reaction of pure quercetin and commercial product chromatographic conditions as follows for $K_2Cr_2O_7$, a mobile phase consisting of methanol (%44) and 0.1 M phosphate buffer (pH 2.15) solution (%52) with 0.4 mL TEA/L (55:45, v/v) and acetonitrile (%4) was used with Water C_{18} column (1.7 μm 2.1 × 100 mm). The retention time of quercetin was 4.47 min with the run time of 6.5 min. The flow rate was 0.2 mL/min, and the injection volume was 1.0 μL. Colon temperature was 25 °C (see Fig. 5a).

For strategy II oxidation reaction of pure quercetin and commercial product chromatographic conditions as follows for KIO_3, suitable resolution and analysis was obtained an isocratic elution using a mobile phase consisting of methanol (%45) and 0.1 M phosphate buffer (pH 2.15) solution (%55) with 0.4 mL TEA/L (55:45, v/v) with Water C_{18} column (1.7 μm 2.1 × 100 mm). The retention time of quercetin was 5.78 min with the run time of 8.0 min. The flow rate was 0.2 mL/min, and the injection volume was 1.0 μL. Colon temperature was 30 °C (see Fig. 5b).

3.4. Analytical validity of methods

For the proposed methods, the linear regression equations were obtained by least squares treatments of calibration data consisting of concentration and response. The performance data and statistical parameters for each method including least squares regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept and slope were listed in Table 1. Useful regression lines with high correlation coefficient valves were obtained from working concentration range between 4 and 20 μg/mL of quercetin (see Table 1). In the estimation of validation parameters, LOD and LOQ correspond to signal-to-noise ratio of 3:1 and 10:1 [33]. In our case, LOD and LOQ values for each method were calculated and presented in same table.

In order to estimate the precision and accuracy of the PARAFAC models, an independent test set of three samples containing quercetin at different concentrations (5, 10 and 20 μg/mL) was prepared and analyzed by the strategy I and strategy II procedures. This recovery procedure was repeated three times for each concentration level. Satisfactory recovery results were obtained from the analysis of the test samples, as shown in Table 2.

To check selectivity of two three-way analysis strategies using kinetic UV spectrometric data sets, added recovery experiments were carried out by spiked technique. Spiked samples were prepared by the standard of quercetin at three levels (low: 4 μg/mL, medium: 8 μg/mL and high: 12 μg/mL) to the solution of dietary supplement product, which contains quercetin at a constant concentration (approximately, 6 μg/mL). At each concentration

Table 2. Recovery results obtained in the test samples by the proposed methods.

Parameter	Strategy (I)			Strategy (II)			UPLC		
Added (μg/mL)	5.0	10.0	20.0	5.0	10.0	20.0	5.0	10.0	20.0
Found (μg/mL)	5.1	10.2	20.3	5.0	10.1	19.9	5.0	10.1	20.6
Recovery (%)	102.7	101.8	101.4	99.6	101.3	99.7	100.1	101.1	102.1
RSD (%)	0.74	1.64	0.37	0.62	0.78	0.51	2.15	0.94	1.57

RSD: Relative standard deviation.

Table 3. Added recovery results obtained by the application of the proposed methods to the spiked samples.

Real sample	Added (μg/mL)	Strategy		UPLC	Strategy		UPLC	Strategy		UPLC
		(I)	(II)		(I)	(II)		(I)	(II)	
		Found (μg/mL)			Added recovery (%)			RSD (%)		
DSP	4.0	3.99	4.02	4.03	99.7	100.5	100.8	1.11	1.57	1.16
DSP	8.0	8.14	8.08	8.02	101.8	101	100.3	1.19	2.45	1.87
DSP	12.0	12.34	12.23	11.91	102.8	101.9	99.3	1.88	1.88	1.02

RSD: Relative standard deviation.
DSP: Dietary supplement product, which contains quercetin at a constant concentration (approximately, 6 μg/mL).

level, the sample was prepared in triplicate. After the oxidation of quercetin in the spiked sample with $K_2Cr_2O_7$, the kinetic UV spectra were recorded and processed by PARAFAC. Then added recovery with relative standard deviation was calculated by subtracting the constant amount of quercetin (in dietary supplement product) from the recovery amount for the sum of the added standard and constant amount of quercetin, taken from commercial dietary supplement product. The assay results for added recoveries and corresponding standard deviations were illustrated in Table 3. Similar procedure was carried out by using the oxidation of quercetin with KIO_3. The assay results for added recovery were listed in the same table. The sample matrix found in commercial dietary supplement product do not show any interference on the analysis of quercetin in effervescent tablets. The accuracy of the method was tested for both strategies by the analysis of synthetic mixtures.

3.5. Method applicability

The applicability of the methods was estimated by applying strategy I and strategy II to the quantification of quercetin in a commercial dietary supplement product. As described above in Strategy I procedure, three-dimensional kinetic UV spectra were obtained, between 225.0 and 545.2 nm (for every 0.2 nm) and 0.0–20.0 min (for every 5 min), by monitoring the oxidation of quercetin in commercial samples by $K_2Cr_2O_7$ as shown in Fig. 1b. In the similar manner, in the strategy II implementation, three-dimensional kinetic UV spectral plots of the commercial samples in the range of 234.0–460.0 nm (for every 0.2 nm) and 0.0–20.0 min (for every 5 min) were recorded by the observation of quercetin oxidation by KIO_3 as given in Fig. 2a and b. The kinetic UV data matrices for the strategy I and the strategy II were obtained from the above mentioned three-dimensional kinetic spectra, and they were stacked to form three-way data arrays. In order to estimate the content of quercetin in the analyzed samples, the PARAFAC model was applied to the decomposition of three-way arrays into a set of trilinear components corresponding to three different profiles (spectra, kinetic curves and relative concentration) of chemical species in the kinetic reaction. The estimated relative concentration profiles were displayed in Fig. 3a (III) and 3b (III) for strategy I using the oxidizing agent, $K_2Cr_2O_7$ and for strategy II involving the use of KIO_3. The individual contributions of the chemical species in spectral and time profiles were already explained. The relative concentration profile obtained was used for the quantitative estimation of quercetin in commercial dietary supplement product as well as validation samples. For quantifying purposes, the calibration equations were obtained by the linear regression of actual concentration on the estimated relative concentration (see Table 1). The concentration of quercetin in real commercial samples was computed by means of the above calibration equations, and the assay results for strategy I and strategy II applications were indicated in Table 4.

Table 4. Determination results obtained by the application of the proposed methods to commercial samples.

Experiment number	Quercetin content (mg/tablet)		
	Strategy (1)	Strategy (II)	UPLC
1	60.05	60.00	60.75
2	60.55	60.05	60.05
3	60.40	60.50	62.00
4	61.00	60.25	61.40
5	60.90	61.25	61.25
Mean	60.58	60.41	61.09
SD	0.77	1.01	1.47
RSD	0.63	0.83	1.20

SD: Standard deviation.
RSD: Relative standard deviation.
Label claim: 60.0 mg quercetin/tablet.

When direct spectrophotometric measurement was applied to the analysis of the same real samples, it was observed that the detection results were lower than the strategy I and II approaches. These undesirable results are due to the spectral interference of excipients in the direct spectrophotometric analysis of the related commercial product. This showed that the improved strategies based on the PARAFAC analysis of the kinetic UV absorbance measurements were reasonable approaches for the analysis of quercetin in dietary supplement product, which was a real complex sample. In addition, to compare the strategy I and strategy II results, the newly developed UPLC method was used for the assay of the same real samples and its analysis results were presented in Table 4.

To compare the means of the assay results obtained by applying strategy I, strategy II and UPLC to the analysis of quercetin in dietary supplement product, the statistical analysis was performed by using one-way ANOVA test (see Table 5). The calculated F-value and calculated *p*-value at the 5% confidence level ($p = 0.05$) were lower than the critical F-value (3.8853) and greater than $p = 0.05$, respectively. It was observed that there was no significant difference between the assay results of the methods (strategy I, strategy II and UPLC). As a consequence, the determination results were found to be comparable for chemometric strategies and UPLC approaches.

Table 5. One-way ANOVA test results for the mean of results obtained by applying the methods to commercial samples.

Source of Variation	SS	df	MS	F cal	*p*-value	F crit
Between Groups	4.9982	2	2.4991	2.0009	0.1779	3.8853
Within Groups	14.9879	12	1.2490			
Total	19.9861	14				

SS: Sum of squares.
df: Degree of freedom.
MS: Mean squares.
p-value: Calculated *p*-value.
F cal: Calculated F-value.
F crit: Critical F-value or tabulated F-value.

4. Conclusions

In this research, two novel analytical strategies with a three-dimensional perspective were improved by using the PARAFAC application to the kinetic UV absorbance data tensors for spectral identifying, kinetic monitoring and quantifying of quercetin in samples, which contain also other chemical species in two different kinetic reactions of the analyte with the oxidizing agents ($K_2Cr_2O_7$ and KIO_3). In spite of the complexity of the reaction media of both strategies I and II, individual signals of components in the resolved spectral, time (kinetic) and relative concentration profiles provided opportunities for the simultaneous evaluation of quercetin within three different frameworks. This was thanks to second-order advantage of three-way analysis approach without using tedious efforts and chemical pretreatment. When the proposed strategies were compared to the newly developed UPLC method, the assay results were found to be comparable for the analysis of quercetin in commercial dietary supplement product. However, due to the interference of the sample matrix, successful analysis results could not be obtained by the application of the direct UV spectrophotometric measurement method to the same commercial samples. The improved analytical strategies can be used as optic sensors for monitoring the kinetic oxidation reaction and quantifying the content of the analyte in commercial dietary supplement samples in the presence of the overlapping spectral bands of the reactants, product and unknown species.

Conflict of interest

All authors declare no conflicts of interest.

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References

[1] Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, et al. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J Funct Foods 2018;40:68–75.
[2] Patel RV, Mistry BM, Shinde SK, Syed R, Singh V, Shin H-S. Therapeutic potential of quercetin as a cardiovascular agent. Eur J Med Chem 2018;155:889–904.

[3] Tang Q, Ji F, Wang J, Guo L, Li Y, Bao Y. Quercetin exerts synergetic anti-cancer activity with 10-hydroxy camptothecin. Eur J Pharmaceut Sci 2017;109:223–32.

[4] Wong G, He S, Siragam V, Bi Y, Mbikay M, Chretien M, et al. Antiviral activity of quercetin-3-b-O-D-glucoside against Zika virus infection. Virol Sin 2017;32:545–7.

[5] Mlcek J, Jurikova T, Skrovankova S, Sochor J. Quercetin and its anti-allergic immune response. Molecules 2016;21:623. https://doi.org/10.3390/molecules21050623.

[6] Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 2007;45:2179–205.

[7] Dmitrienko SG, Kudrinskaya VA, Apyari VV. Methods of extraction, preconcentration, and determination of quercetin. J Anal Chem 2012;67(4):299–311.

[8] Karmakar A, Singh B. Spectroscopic analysis and theoretical investigation of hydrogen bonding interaction of quercetin with different acceptor molecules. J Mol Struct 2019;1180: 698–707.

[9] Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, et al. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J Funct Foods 2018;40:68–75.

[10] Karrat A, Palacios-Santander JM, Amine A, Cubillana-Aguilera L. A novel magnetic molecularly imprinted polymer for selective extraction and determination of quercetin in plant samples. Anal Chim Acta 2022;1203:339709.

[11] Yao Y-Y, Zhang L, Wang Z-F, Xu J-K, Wen Y-P. Electrochemical determination of quercetin by self-assembled platinum nanoparticles/poly (hydroxymethylated-3,4-ethylenedioxylthiophene) nanocomposite modified glassy carbon electrode. Chin Chem Lett 2014;25(4):505–10.

[12] Andres S, Pevny S, Ziegenhagen R, Bakhiya N, Schafer B, Hirsch-Ernst KI, et al. Safety aspects of the use of quercetin as a dietary supplement. Mol Nutr Food Res 2018;62(1): 1700447.

[13] Vida RG, Fittler A, Somogyi-V'egh A, Poor M. Dietary quercetin supplements: assessment of online product informations and quantitation of quercetin in the products by high-performance liquid chromatography. Phytother Res 2019;33(7):1912–20.

[14] Quercetin D'Andrea G. A flavonol with multifaceted therapeutic applications? Fitoterapia 2015;106:256–71.

[15] Zazeri G, Povinelli APR, Pavan NM, Daniella Romano de Carvalho DR, Cardoso CL, Ximenes VF. Experimental studies and computational modeling on cytochrome c reduction by quercetin: the role of oxidability and binding affinity. J Mol Struct 2021;1244:130995.

[16] Johnson MK, Loo G. Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA. Mutat Res 2000;459:211–8.

[17] Barnes JS, Schug KA. Oxidative degradation of quercetin with hydrogen peroxide using continuous-flow kinetic electrospray-ion trap-time-of-flight mass spectrometry. J Agric Food Chem 2014;62:4322–31.

[18] Makris DP, Rossiter JT. An investigation on structural aspects influencing product formation in enzymic and chemical oxidation of quercetin and related flavonols. Food Chem 2002;77:177–85.

[19] Makris DP, Rossiter JT. Hydroxyl free radical-mediated oxidative degradation of quercetin and morin: a preliminary investigation. J Food Compos Anal 2002;15:103–13.

[20] Wang C, Zuo Y. Ultrasound-assisted hydrolysis and gas chromatography-mass spectrometric determination of phenolic compounds in cranberry products. Food Chem 2011;128:562–8.

[21] Soylak M, Ozdemir B, Yilmaz E. An environmentally friendly and novel amine based liquid phase microextraction of quercetin in food samples prior to its determination by UV-vis spectrophotometry. Spectrochim Acta Part A Mol Biomol Spectrosc 2020;243:118806.

[22] Chen G, Zhang H, Ye J. Determination of rutin and quercetin in plants by capillary electrophoresis with electrochemical detection. Anal Chim Acta 2000;423(1):69–76.

[23] Molinelli A, Weiss R, Mizaikoff B. Advanced solid phase extraction using molecularly imprinted polymers for the determination of quercetin in red wine. J Agric Food Chem 2002;50:18041808.

[24] Xu S, Chen L, Ma L. Fluorometric determination of quercetin by using graphitic carbon nitride nanoparticles modified with a molecularly imprinted polymer. Microchim Acta 2018; 185:492.

[25] Zhao P, Ni M, Xu Y, Wang C, Chen C, Zhang X, et al. A novel ultrasensitive electrochemical quercetin sensor based on MoS_2-carbon nanotube@graphene oxide nanoribbons/HS-cyclodextrin/graphene quantum dots composite film. Sensor Actuator B Chem 2019;299:126997.

[26] Pejic N, Kuntic V, Vujic Z, Micic S. Direct spectrophotometric determination of quercetin in the presence of ascorbic acid. Il Farmaco 2004;59:21–4.

[27] Pavun L, Durđević P, Jelikić-Stankov M, Dikanović D, Ćirić A, Uskoković-Marković S. Spectrofluorimetric determination of Quercetin in pharmaceutical dosage form. Maced J Chem Chem Eng 2014;33(2):209–15.

[28] Wang FM, Yao TW, Zeng S. Determination of quercetin and kaempferol in human urine after orally administrated tablet of ginkgo biloba extract by HPLC. J Pharm Biomed Anal 2003;33:317–21.

[29] Kumar S, Lather V, Pandita D. Stability indicating simplified HPLC method for simultaneous analysis of resveratrol and quercetin in nanoparticles and human plasma. Food Chem 2016;197:959–64.

[30] Martelo-Vidal MJ, Vázquez M. Determination of polyphenolic compounds of red wines by UV-VIS-NIR spectroscopy and chemometrics tools. Food Chem 2014;158:28–34.

[31] Hassan HNA, Barsoum BN, Habib IHI. Simultaneous spectrophotometric determination of rutin, quercetin and ascorbic acid in drugs using a Kalman Filter approach. J Pharm Biomed Anal 1999;20:315–20.

[32] Andersson CA, Bro R. The N-way toolbox for MATLAB. Chemometr Intell Lab Syst 2000;52:1–4.

[33] The United States pharmacopeia 30th edition, the national formulary. In: 25th edition 'the official compendia of standards' United States pharmacopeia convention, INc.; 2007. Asian Edition, Washington, D.C.