Cumhuriyet Science Journal
e-ISSN: 2587-246X
ISSN: 2587-2680
Cumhuriyet Sci. J., 42(3) (2021) 629-637
http://dx.doi.org/10.17776/csj.937421

Synthesis of new solid phase sorbent for sensitive spectrophotometric determination of Quercetin

Songül ULUSOY [1,*]

[1] *Cumhuriyet University, Department of Pharmacy, Vocational School of Health Service, Sivas 58140, TURKEY*

Abstract

Sensitive and easy applicable analysis of Quercetin molecules was executed whereby solid phase extraction (SPE) and molecular spectrophotometric determination. For the determination of quercetin, an accurate, economical, time-saving and a new environmentally friendly method has been developed with a simple spectrophotometer device available in every laboratory. As a solid-phase sorbent, a new polymer nanocomposite has been synthesized and characterized by Fouirer Transform Infrared Spectroscopy (FT-IR) and Scanned Electron Microscopy (SEM). Experimental solid phase extraction variables have been investigated and optimized step by step, such as pH, adsorption time and desorption conditions, etc. According to optimization results, desorption of quercetin molecules was carried out by 2-propanol solvent and absorbance of eluent solutions were measured as systematic at 255 nm and at 370 nm. However, the best results were obtained at 370 nm wavelength. Therefore, all measurements were made at 370 nm. (LOD) means that the detection limit and (LOQ), the quantitation limit values for quercetin were calculated as 5.97 ng $mL^{-1}$and 17.91 ng mL^{-1}, respectively. Standard addition and recovery experiments were performed as an indicator of the accuracy of the method. The method developed was effectively conducted to model solutions.

Article info

History:
Received: 15.05.2021
Accepted: 07.09.2021

Keywords:
Quercetin,
Solid phase extraction,
Polymeric adsorbent,
Analytical method development.

1. Introduction

Flavonoid molecules in food samples have a long history of natural sources of antioxidants. The determination of such molecules in real samples attracts much attention in many countries. Main properties of these compounds can be displayed such as anti-carcinogenic, anti-allergic, anti-inflammatory, and antiviral action[1]. Quercetin, which is systematically named as (3,5,7,3′, 4′-pentahydroxyflavone) by IUPAC, is in the flavonoid class of bioflavonoids. Molecular formula of quercetin was given in Figure 1.

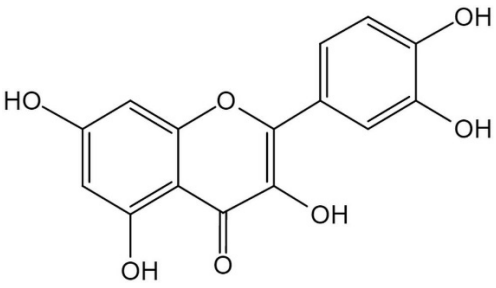

Figure 1. Molecular structure of quercetin

Quercetin is mostly existed in various samples such as, coffee, tea, vegetables, seeds, fruits, fern and natural dyes. Flavonoids are actually bioflavonoids that are composed of more than 6000 different species found in every plant. It gives plants bright yellow, orange and red colors that dazzle our eyes. Most flavonoids act as antioxidants in the human body. With these functions, they prevent the cells from being damaged by neutralizing the excessively reactive molecules containing oxygen. They show a 2-phenyl benzopyron structure (C6-C3-C6) with 15 C atoms. They are considered as polyphenolic compounds due to their structure. Since their skeletal structures are different, they have derivatives such as flavone, flavonol, flavonone, biflavonoid, chalcone. Flavonoids have been reported to have other properties than antioxidant properties. These properties can be summarized as follows; antitumor effect, antiviral effect, antithrombotic effect, anti-inflammatory effect, antiallergic effect, protection effect from atherosclerosis and coronary heart diseases[2,3].

Flavonoids constitute one of the most characteristic classes of compounds found in most plant species. The vast majority of flavonoids are easily recognized as

*Corresponding author. *e-mail address: sonulusoy@yahoo.com*

flower pigments in the flowering plant family but are not always floral pigments. Analysis of flavonoids has interested much attention by many researchers from different and interdisciplinary so like that chemistry, pharmacy, medicine, biology area etc. Quercetin, a polyphenol belonging to the class of flavonoids, is categorized as flavonol, one of the subclasses of flavonoid compounds.[4]. Thanks to its superior chemical and biological properties, quercetin has proven to have an important role in the treatment of many diseases such as ulcers, allergies, diabetes, cancer, cardiovascular and inflammatory diseases. [5,6]. Quercetin molecules acts as a free-radical scavenger and shows a useful antioxidant properties for human health [7]. Various analytical methods are used for analysis of quercetin molecules in real samples such as spectrophotometric [2], chromatographically [8] and electroanalytical methods [9]. Mostly, a pre-concentration method is combined with these technical[10]. Simple and reliable separation and pre-concentration methods are generally preferred for the sensitive determination of naturally occurring biomolecules in samples. The primary challenge of this type of analysis is to assess the success of the pretreatment method. Solid phase extraction approaches are mostly preferred owing to easy applicable properties. The major advantages of combining this approach with the conventional analytical method are the ability to determine the trace analyte without the use of complicated or expensive tools. The richest food sources of natural antioxidant quercetin are broccoli , grapes, apples, citrus fruits ,strawberries, cherries, onions ,capers, buckwheat, green leafy vegetables, hazelnuts and black tea[11]. By cause of the complex matrix and the trace amount of quercetin in real samples, a good sample preparation and method development is needed to identify target molecules. The main challenge in this procedure is to do the preconcentration method correctly. The better this step, the more successful the enrichment process. Magnetic solid phase extraction is preferred as a simple method to concentrate target molecules prior to the detection step. [12]. For this, it is applied by interacting with target molecules using magnetic solid phase extraction, which is one of the sample preparation techniques, before the determination process. [13]. Newly, more and more of these utilizations have given the use of hybrid nanoparticles for the selective extraction of trace species from a complex matrix environment[14–16]. The major advantage of combining this approach with the conventional method of analysis is the ability to determine the trace analyte without the use of complicated or costly tools[17].

In the light of the information described above, as part of current study, a method for the adsorption and enrichment of quercetin in model solutions with a newly developed polymeric material was developed with the help of UV-VIS spectrophotometer. The developed method is applied to model solutions. A method has been developed for the separation and enrichment of quercetin in model solution and its determination by UV-VIS spectrophotometer. In this method, a new polymeric material was synthesized and characterization studies were carried out. By optimizing the necessary analytical parameters (pH, adsorption time, extraction solvent volume, etc.), recovery studies were carried out for the quantitative analysis of quercetin.

2. Materials and Methods

2.1. Apparatus

A spectrophotometer (Shimadzu, UV-Visible 1800, Japan) equipped with a 1 cm quartz cell was used for absorbance measurements. This spectrophotometer has in the wavelength range of 190–1100 nm. A pH meter with a glass electrode (Hanna, Germany) was used to measure the pH values. A centrifuge (Elektromag centrifuge, Turkey) was used to accelerate the phase separation. Orbital shaker (Elektromag) was used to achieve homogenization between the analyte and the synthesized material. FT-IR analysis of developed material was carried out by using the FT-IR spectrophotometer (Bruker) for checking of functional groups in the range of 400–4000 cm^{-1}. The surface morphology of solid phase material was observed with a scanning electron microscope (TESCAN MIRA3 XMU, FEG-SEM, Brno, Czechia).

2.2. Reagents

Ultrapure Milli-Q water (18.2 MΩ cm, Millipore Corporation, Turkey) was used in all of the experiments. A 500 μg mL^{-1} of Quercetin solution (Sigma, St. Loius, MO, USA) was prepared by dissolving quercetin in isopropanol 0.02 M Britton-Robinson (BR) buffer solution was used to work at required pH values. Acryl amide(AA), N,N′-methylenebisacrylamide , ammonium persulphate, and fuchsin acid (FA) were also used at analytical grade. The chemicals contained in this buffer are as follows. 0.024 M H_3BO_3 (Merck), 0.02 M H_3PO_4 (Merck) and 0.02 M CH_3COOH (Merck) that has been set to the wanted pH with 0.1 M NaOH.

2.3. Synthesis of solid phase material

A new adsorbent was synhtezied for using as solid phase sorbent(PAA@FA). It is widely known polymeric material with some minor modifications.

PAA@FA was prepared by a chemical reaction by means of adding fuchsin acid (FA) during poly acryl amide (PAA) formation. It is well known grafting procedure for this type[18,19] materials. Briefly, 200 mg of FA were dissolved completely in the solution including 5 g of acrylamide and 0.5 g of cross-linker (N,N′-methylenebisacrylamide) in 10 mL of water. Then, the mixture was kept in ultrasonic water bath for 10 min and mixture at 500 rpm on a magnetic stirrer. For starting of polymerization reaction, 0.2 g of ammonium persulphate was transferred to this solution immediately. After the polymerization reaction was sustained by adding 100 µL of N,N,N′,N′-tetramethylethylenediamine (TEMED) at room temperature. PAA@FA was washed with ultra-pure water for five times and ethanol for 2 times in order to remove non-grafted reagents. The collocted composite was dried at 40 °C in a vacuum oven.

2.4. The solid phase extraction procedure

10 ml of fractional sample containing Quercetin (within 10- 1200 ng mL^{-1}) was located in a falcon tube. Then 1.0 mL of pH: 4.00 buffer, 50 mg of adsorbent (PAA@FA) were suplemented and completed to 10 mL with ultra-pure water. The latest solution was retained on an orbital shaker at 100 revolutions per minute for 40 minutes. At the end of this period, the adsorbent was isolated from the aqueous medium by centrifugation at 4000 revolutions per minute for 5 minutes. The watery phase was ejected with the help of an injector and the adsorbent phase was gathered at the base of the tube. And then, 400 µL of 2-propanol was joined later, stripping adsorbed quercetin molecules using a wormhole for 30 s prior to spectrophotometric detection at 370 nm. Another blank solution was subjected to the same process. Finally, the quantity of Quercetin was determined using either the calibration curve obtained directly from the spectrophotometer or the standard addition method.

2.5. Application to model solutions

The suggested method was practiced to model solutions including quercetin at various concentrations. The prepared model solutions were filtered through Whatman grade No. 40 filter paper. 20 mL of this solution was treated under the developed method.

3. Results and Discussion

3.1. Selection of working wavelength

Figure 2 exhibits the absorption spectra of Quercetin after SPE for five different concentration levels. The obtained results showed that maximum absorbance occurs at 255 and 370 nm. As is shown, the absorbance of target molecules increased progressively with concentration at both wavelengths.

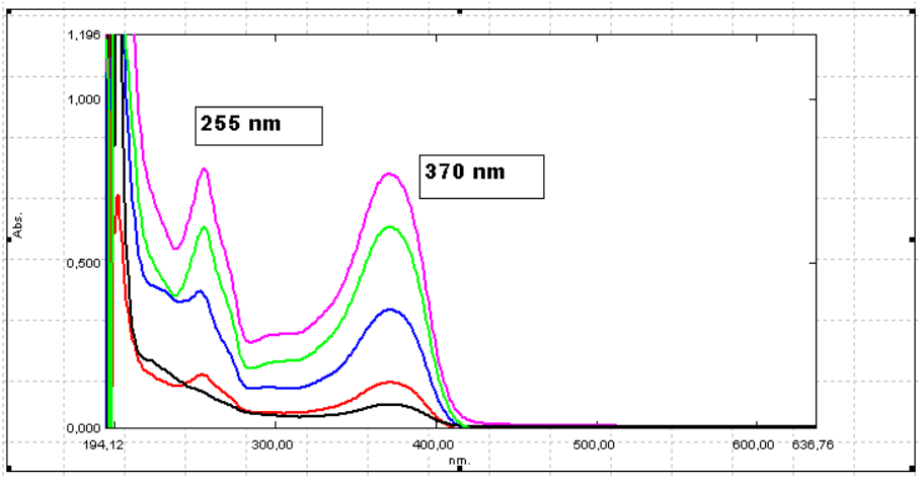

Figure 2. Absorption spectrum of Quercetin solutions in different concentrations after SPE

3.2. Characterization of synthesized material

The FT-IR spectra of the used polymeric material (PAA@FA) was illustrated in Figure 3. The spectrum of PAA showed a broad band of NH_2 at 3300 cm^{-1}, peaks of carboxyl and amide groups ($CONH_2$) at 1600–1700 cm^{-1}, and a C-N stretch at 1000–1200 cm^{-1} [20]. There was no significant difference between PAA and FA because the concentration of the grafted molecules was very low. Only, the grafting procedure can be provided by increasing peak at 2800 cm^{-1}.

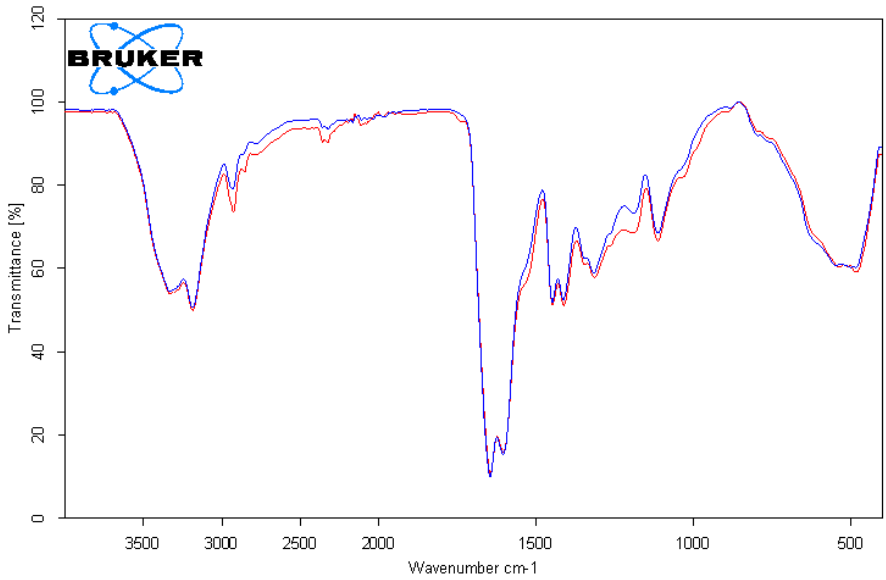

Figure 3. FTIR spectrum of new developed polymeric material (Red: PAA, Blue: PAA@FA)

SEM images of PAA@FA was shown in Fig. 4. The surface morphology of PAA@FA is clearly seen in this figure with different focusing centers. The image of PAA@FA demonstrates a porous and bittie surface structure, while it displays an in-order surface and smoother structure. Adsorbent surface morphology is suitable for adsorption of target molecules.

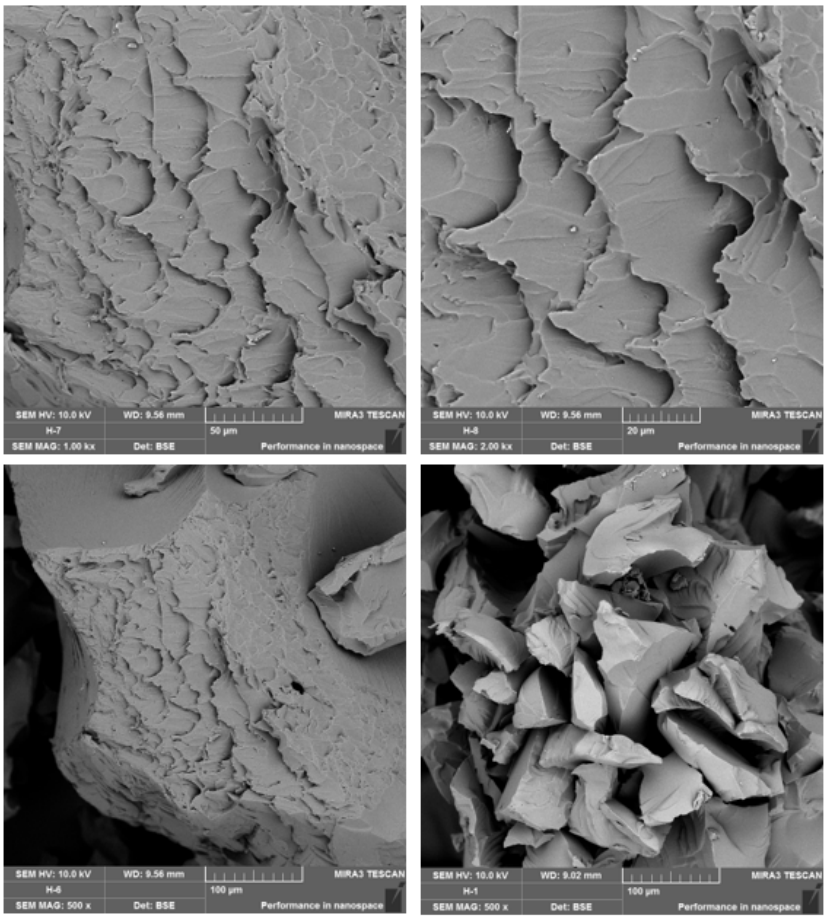

Figure 4. SEM image of new developed polymeric material

3.3. Effect of pH

Considering the pH of the sample solution on both adsorption and enrichment factor, it is of great importance to study this parameter. The effect of pH on extraction efficiency was studied by changing the pH of the sample solution from 2 to 10. The results were shown in Figure 5. The signals of Quercetine increased until pH 4.0 and decreased beyond this pH. Possibly, an increase in pH resulted in a decrease in the transfer of Quercetin molecules. The pKa value of the quercetin is 6.38 [21]. Beyond this value, hydrogen ion seperate from quercetine and the molecule turns into ionized negative charged form. And, this change decrease the extraction efficiency. Then, the next studies were continued by using pH 4.0 buffer. After suitable pH were selected, the concentration of buffer (0.1 M) was studied within the framework 0-2 mL by means of changing volume of buffer. According to experimental results as illustrated Figure 5, better signals were achieved using 1 mL buffer solution.

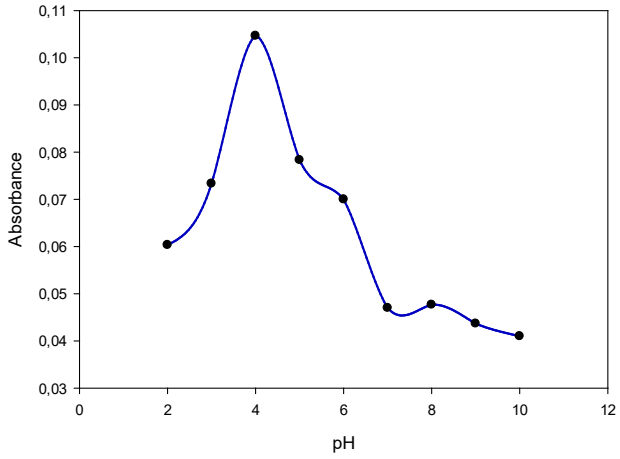

Figure 5. The effect of pH on developed method

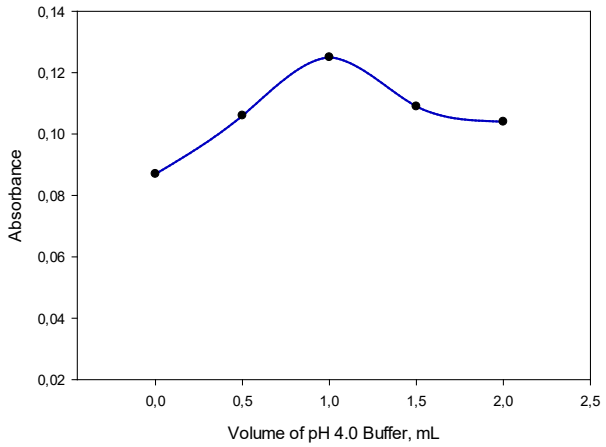

Figure 6. The volume effect of pH on developed method

3.4. Eluent(desorption) type and its volume

The suitable solvent should be chosen for desorption process which directly effects success of pre-concentration method. Therefore, one of the most important steps in SPE experiments is to determine the most ideal solvent for the elution process. In addition, when choosing the eluent, its suitability to the detection system should also be considered. The change of analytical signals by different solvent types on desorbing of Quercetin out of PAA@FA were studied and the results were presented in Figure 7. As shown in the Figure, the best signals of Quercetin after SPE were acquired through isopropanol (2-proponal). In our study, the volume of elution solvent is one of the important parameters to obtain a high preconcentration factor (PF). Because PF decrease while the volume increases due to dilution effect. For this reason, the affect of eluent volume on pre-concentration of Quercetin was examined in the scope of 300-1500 µL. As reflected from Figure 7 the best signal values were acquireed with 600 µL 2-propanol. Consequently, next steps were pursued utilizing this value.

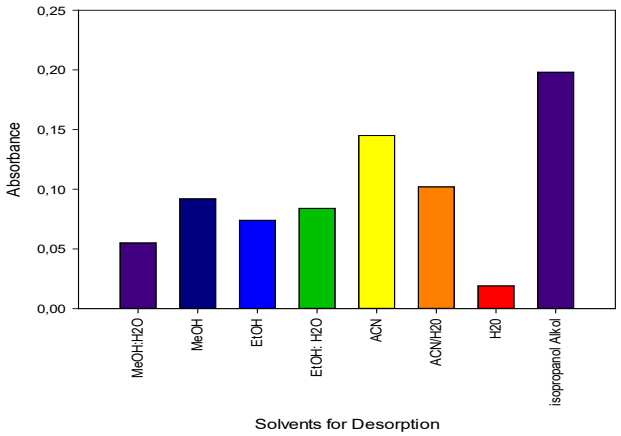

Figure 7. The effect of desorption solvent

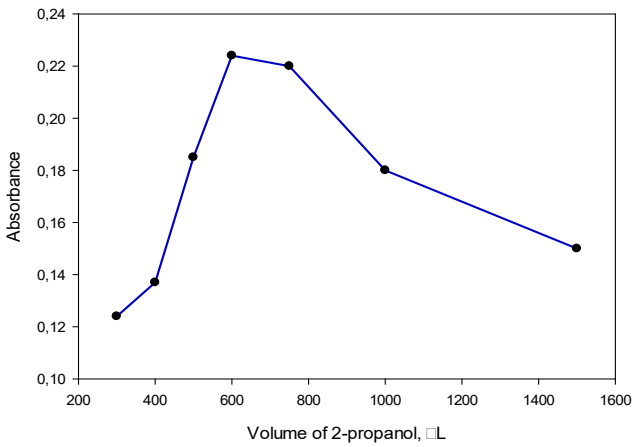

Figure 8. Volume optimization of desorption solvent

3.5. Optimization of adsorption and elution time

Our goal in this optimization step is to ensure that the experimental procedure is completed as soon as possible. The developed method includes two main steps: pre-concentration and determination. As is known, the determination step is a routine spectrophotometric analysis and there is nothing that can be done to shorten this step. For this reason, solid phase extraction experiments, which the enrichment step, were studied and evaluated in terms of time. SPE procedures include adsorption and desorption steps. The adsorption step of SPE was carried out using orbital shaker at room temperature. Secondly, the desorption step of SPE was performed by vortexing at 100 rpm. Time optimization for the adsorption step was investigated in the range of 0 to 100 minutes using 200 ng mL^{-1} Quercetin model solutions. Desorption time was optimized using model solutions in the range of 5-60 s. As seen in Figure 9, 40 minutes is sufficient for complete adsorption of quercetin molecules at a shaking speed of 100 rpm. In addition, 30 seconds of vortexing is sufficient for all of the analyte to pass into the elution solution.

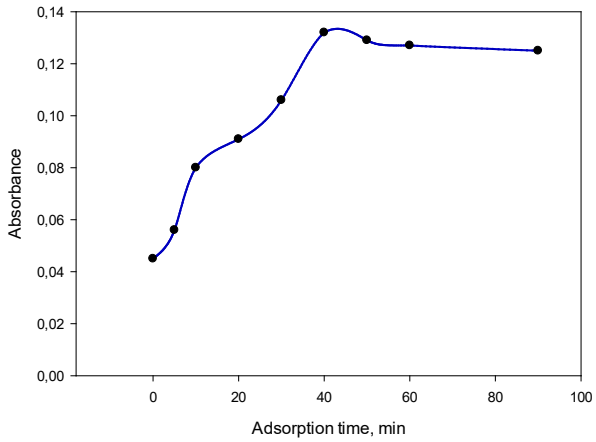

Figure 9. Optimization of adsorption time on the developed method

3.6. Reusability properties of material

One of the most important properties of a new developed material is reusability studies. The analysis cost of a new method is directly affected of new material. In a solid phase extraction method, if sorbent phase can be applied once more, the cost of the analysis decreases. The measure of the reusability of a material shows the robustness of that material.

For this study, the new polymeric material was weighed and run the optimized extraction conditions. After every use, the amount of adsorbed quercetin molecules was compared previous one. The experiments were kept to obtain 5.0 % a change in measured amount of quercetin. It was noticed that there isn't significant change in the signal after 10 times use. Then for each experiment, polymeric material was washed with 2.0 mL isopropanol twice after each use.

3.7. Analytical characteristics

The analytical frameworks of the proposed method were studied step by step that is, LOD and LOQ, linear range ,repeatability ,regression equation and pre-concentration factor. The linearity of method was observed in the range of 20-800 ng mL^{-1}.

LOD and LOQ values were calculated as 5.97 and 17.91 ng mL^{-1}, respectively. The preconcentration factor was determined as a factor of 16.7 because SPE experiment started with 10 mL and final volume of solution was 0.6 mL after SPE. Moreover, the enrichment factor was also found as 36 by calculating the ration between slopes of the calibration plots before and after SPE.

Table 1. Analytical qualities of the presented method .

Parameters	The Values	
	Before SPE	After SPE
Linear range	0.8–5.0 µg mL^{-1}	20–800 ng mL^{-1}
Slope	0.009	0.324
Correlation coefficient (r^2)	0.9928	0.9915
RSD (%)	3.5 (for 1.00 µg L^{-1}, n: 5)	2.8 (for 200 ng mL^{-1}, n: 5)
LOD	0.24 µg L^{-1}	5.97 ng mL^{-1}
LOQ	0.72 µg L^{-1}	17.91 ng mL^{-1}
Preconcentration factor[a]	-	16.7
Enrichment factor[b]	-	36

3.8. Determination of Quercetin in Model Solutions

Application of the submitted method was tested via model solutions. Repeatability, and accuracy values was evaluated by relative standard deviations and recovery values, respectively. Every sample was analyzed for 5 replicate. The results were demonstrated in Table 2. Recovery studies were performed too after having been enriched with samples of known quercetin concentrations of 50 and 100 ng mL^{-1}. RSD % values were calculated as indicator of repeatability of method by considering intraday and interday analysis results.

Table 2. Determination of Quercetin in model solutions (N=5)

Sample	Added Quercetin ng mL^{-1}	Found Quercetin ng mL^{-1}	Recovery %	intraday RSD%	interday RSD%
	-	- -	-	-	-
Model Solution	50.00	50.78 ± 1.45	101.6	2.85	3.65
	100.00	102.93 ± 2.52	102.9	2.45	3.52

3. Conclusions

In this presented study, a new polymeric adsorbent material was firstly synthesized for the enrichment and determination of Quercetin molecules. Solid phase extraction experiments and all necessary optimization processes were performed with this original material. The data obtained as a result of all these optimization processes; A new, economical, very simple and environmentally friendly analytical method has been developed for the separation and enrichment of quercetin molecules in model solutions by UV-spectrophotometric determination. An effective and easily method for determination of Quercetin molecules by means of the SPE. The method offers significant green advantages very little the use of organic solvents in the extracting process. The proposed methodology consists of a simple experimental procedure. The required experimental equipment can simply be provided by each analytical laboratory. A comparison table was shown in table 3. As can be seen, the proposed method has similiar and comparable properties with literature.

Table 3. Comparison of the developed method with other existing methods.

Pre-treatment Procedure	Determination Method	Limit of detection	Pre-concentration factor (PF)or Enrichment factor(EF)	Ref.
Deep eutectic solvent in ultrasound-assisted emulsification microextraction	UV-VIS	18.8 μg L^{-1}	15 (PF)	[2]
Amine-based liquid phase microextraction	UV–VIS	70 μg L^{-1}	26.7 (PF)	[21]
Ultrasonic-assisted restricted access supramolecular solvent-based liquid phase microextraction	UV–VIS	9.93 μg L^{-1}	30 (PF)	[16]
Magnetic solid phase extraction	HPLC-DAD	1.46 ng mL^{-1}	125 (PF) 103.7 (EF)	[14]
Solid Phase Extraction (SPE)	UV–VIS	5.97 ng mL^{-1}	16.7 (PF) 36 (EF)	The Developed Method

The number of methods used in the literature for analysis of quercetin molecules is generally used chromatographicall approeches. But, it is well known, every laboratury does not have this facilities. Spectrohotomeric methods and the used equipment are available in almost research laboratory. For this reason, the important of developed method can be understood better.

Conflicts of interest

There is no conflict of interest: The author state that did not have conflict of interests

References

[1] Kurzawa M., Determination of quercetin and rutin in selected herbs and pharmaceutical preparations, *Anal. Lett.,* 43 (2010) 993–1002.

[2] Kanberoglu G.S., Yilmaz E., Soylak M., Application of deep eutectic solvent in ultrasound-assisted emulsification microextraction of quercetin from some fruits and vegetables, *J. Mol. Liq.,* 279 (2019) 571–577.

[3] Zhiltsova E.P., Ibatullina M.R., Lukashenko S.S., Kutyreva M.P., Zakharovac L.Y., Spectrophotometric study of quercetin in metallomicellar solutions of 1-hexadecyl-4-aza-1-azoniabicyclo[2.2.2] octane bromide complex with copper dibromide, *J. Mol. Liq*., 249 (2018) 716–722.

[4] Ranjbar Banforuzi S., Hadjmohammadi M.R., Two-phase hollow fiber-liquid microextraction based on reverse micelle for the determination of quercetin in human plasma and vegetables samples, *Talanta,* 173 (2017) 14–21.

A. Murakami H., Ashida J., Terao, Multitargeted cancer prevention by quercetin, *Cancer Lett*., 269 (2008) 315–325.

[5] Boots A.W., Haenen G.R.M.M., Bast A., Health effects of quercetin: From antioxidant to nutraceutical, *Eur. J. Pharmacol*., 585 (2008) 325–337.

[6] Lobo V., Patil A., Phatak A., Chandra N., Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacogn. Rev*., 4 (2010) 118.

[7] Jiang H., Engelhardt U.H., Thräne C., Maiwald B., Stark J., Determination of flavonol glycosides in green tea, oolong tea and black tea by UHPLC compared to HPLC, *Food Chem*., 183 (2015) 30–35.

[8] Xiao P., Zhao F., Zeng B., Voltammetric determination of quercetin at a multi-walled carbon nanotubes paste electrode, *Microchem. J*., 85 (2007) 244–249.

[9] Chafer A., Pascual M.C., Salvador A., Berna A., Supercritical fluid extraction and HPLC determination of relevant polyphenolic compounds in grape skin, *J. Sep. Sci.,* 28 (2005) 2050–2056.

[10] Wu H., Chen M., Fan Y., Elsebaei F., Zhu Y., Determination of rutin and quercetin in Chinese herbal medicine by ionic liquid-based pressurized liquid extraction-liquid chromatography-chemiluminescence detection, *Talanta,* 88 (2012) 222–229.

[11] Ranjbari E., Biparva P., Hadjmohammadi M.R., Utilization of inverted dispersive liquid-liquid microextraction followed by HPLC-UV as a sensitive and efficient method for the extraction and determination of quercetin in honey and biological samples, *Talanta,* 89 (2012) 117–123.

[12] Ulusoy H.İ., Applications of magnetic nanoparticles for the selective extraction of trace species from a complex matrix, 1st ed., New York: Nova Scientific Publishers, (2017).

[13] Ulusoy S., Yilmaz E., Erbas Z., Ulusoy H.I., Soylak M., Trace analysis of quercetin in tea samples by HPLC-DAD system by means of a new nanocomposite including magnetic core-shell, *Sep. Sci. Technol*., (2019).

[14] Şenol Z.M., Şimşek S., Ulusoy H.İ., Mahmood A., Kaya S., Insight from adsorption properties of Xylidyl Blue embedded hydrogel for effective removal of uranyl: Experimental and theoretical approaches, *Polym. Test*., (2020).

[15] Memon Z.M., Yilmaz E., Soylak M., Multivariate statistical design optimization for ultrasonic-assisted restricted access supramolecular solvent-based liquid phase microextraction of quercetin in food samples, *J. Iran. Chem. Soc*., 14 (2017) 2521–2528.

[16] Karaca E., Ulusoy S., Morgül U., Ulusoy H.İ., Development of Analytical Method for Sensitive Determination of Streptozotocin based on Solid Phase Extraction, *Cumhur. Sci. J.,* 41 (2020) 826–831.

[17] Ulusoy H.I., Şimşek S., Removal of uranyl ions in aquatic mediums by using a new material: Gallocyanine grafted hydrogel, *J. Hazard. Mater*., 255 (2013) 397-405.

[18] Şimşek S., Şenol Z.M., Ulusoy H.İ., Synthesis and characterization of a composite polymeric material including chelating agent for adsorption of uranyl ions, *J. Hazard. Mater.,* 338 (2017) 437–446.

[19] Ulusoy H.T., Şimşek S., Removal of uranyl ions in aquatic mediums by using a new material: Gallocyanine grafted hydrogel, *J. Hazard. Mater.* 254–255 (2013) 397-405.

[20] 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*., 243 (2020) 118806.