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Preconcentration of flavonoids on polyurethane foam and their direct determination by diffuse reflectance spectroscopy

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ABSTRACT

Sorption preconcentration of flavonoids quercetin, rutin, chrysin, morin, naringenin and naringin on polyurethane foam was investigated. Several parameters that could affect the preconcentration efficiency were evaluated. The preconcentration efficiency is more than 75% for all the flavonoids except for those that are carbohydrate substituted (preconcentration efficiency less than 11%). This can be used for the separation of these two types of flavonoids. An ability of some flavonoids to absorb light in PUF phase allows their direct determination by diffuse reflectance spectroscopy. Validation of calibration linearity, reproducibility, limits of detection and quantification was performed. The method developed allows to determine flavonoids with detection limits 0.01–0.2 μg mL^{-1}. The method was utilized for the determination of quercetin in some plant extracts.

1. Introduction

Flavonoids (FL) are widely spread natural heterocyclic antioxidants. Their molecules contain two benzene rings related via a three-carbon fragment which usually forms a cycle by oxygen atom. Flavonoids possess a wide spectrum of biological effects: antioxidant, immunopotentiating, anticancer, cardio-, hepato-, antiallergic, antiinflammatory and antivirus activities [1–3].

Numerous pharmaceutical preparations containing flavonoids are available in the market. As a result, a simplified analytical technique is required for pre-concentration and determination of flavonoids in plants, food, biological fluids and pharmaceutical dosage forms. The demands being made to the analysis techniques of natural objects and biological samples, characterized by the complex composition and low content of flavonoids as well as pharmaceutical dosage forms containing high amounts of these substances, vary essentially.

The recent methods applied for the pre-concentration and determination of flavonoids include mostly high performance liquid chromatography (HPLC) [4–6], HPLC–mass spectrometry [7–9] and capillary electrophoresis [10,11]. These methods occupy leading positions in the determination of flavonoids in plants, food and biological fluids. They provide high sensitivity for the assay; yet, they also have disadvantages, such as complexity of operation, high reagent consumption and high cost. Some other techniques such as voltammetry [12–14], chemoluminescence [15], spectrophotometry [16,17] and microsequential flow injection analysis [18] have also been employed for the determination of flavonoids in samples of different kinds.

Sample preparation is a key step, preceding the determination of flavonoids in real objects. Nowadays solid-phase extraction (SPE) is becoming more popular because it requires less organic solvent, is easy to implement, allows high sample throughputs and, in general, is used with good results. Flavonoids can easily be preconcentrated by SPE using any reversed-phase material such as alkyl-modified silica [19,20] or polymeric sorbents [19–22]. A relatively new SPE method uses a molecularly imprinted polymer (MIP) as the sorbent [23–25]. MIPs, typically, are highly selective for the target analyte and usually have good mechanical and thermal stabilities.

The main goal of this work was to investigate sorption of flavonoids on polyurethane foam (PUF) followed by their direct determination by diffuse reflectance spectroscopy in the visible region. Among the many available materials that are used for solid phase extraction of organic compounds, PUF has such advantages as smaller resistance to fluid passage, very low cost and stability in acidic or basic media [26]. A variety of procedures to obtain a colored organic compound in the sorbent phase are proposed for the determination of different analytes by preconcentration with PUF and subsequent determination by diffuse reflectance spectroscopy. One approach is based on the sorption of colored compounds formed by a test component and the derivatizing agent in the analyzed solution. This approach has been applied to the determination of phenols [27], 1-naphthol [28] and gallic acid [29] in the forms of their colored azoderivatives as well as to the determination of cationic [30] and anionic

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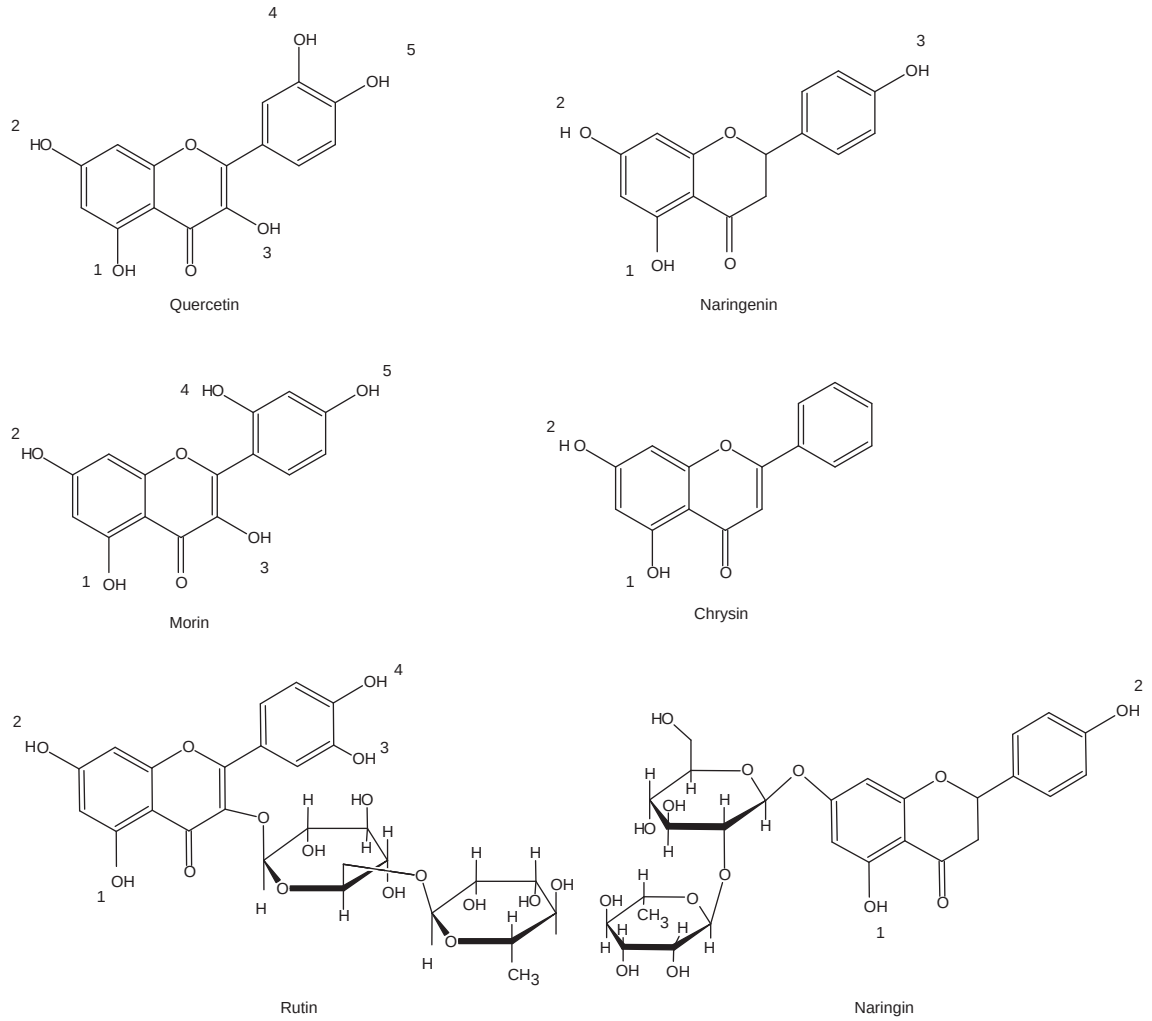

Fig. 1. Chemical structures of flavonoids used in this study.

[31] surfactants in the forms of their ionic associates with colored counterions. Another approach is based on chemical reaction between the substance to be determined and the functional groups of PUF. We have previously shown that terminal toluidine groups like aromatic amines react with several substances giving colored products [32–36]. And finally, colored organic substances can be determined in the matrix of PUF without carrying out the reaction of derivatization; however, the examples illustrating the possibility of such combinations are very limited [37] since the range of such substances is extremely limited. In our study we have suggested that some colored flavonoids could be determined in the phase of PUF by diffuse reflectance spectroscopy.

2. Experimental

2.1. Materials

All chemicals used in this study were at least of analytical reagent grade. Quercetin was purchased from Sigma, rutin, chrysin, morin, naringenin and naringin were purchased from Acros. The chemical structures of these compounds are given in Fig. 1. The solutions were always prepared with ultrapure water purified by a Simplicity Milli-Q System (Millipore, Bedford, USA). Stock standard solutions of flavonoids (0.001–0.01 mol L^{-1}) were prepared in ethanol. The working solutions were prepared by appropriate dilution of the stock solutions with double distilled/deionized water.

The herb (*Monarda didyma L*), the dietary grape polyphenol concentrate "*Enoant*" and ethanol tincture of hawthorn were purchased from a local pharmacy, and the yellow onion was purchased from a local market.

Open-cell polyether type PUF (5–30, Ukraine) was used. PUF tablets (16 mm diameter, 5 mm-thick) were cut from a commercially available polymer sheet. The mass of each PUF tablet was 0.020 ± 0.001 g. The tablets were washed with acetone, air-dried, and stored in the dark.

2.2. Apparatus

Diffuse reflectance spectra and diffuse reflectance values were measured on a "Spectroton" colorimeter (NPO Khimavtomatika, Chirchik, Uzbekistan). This device can measure diffuse reflection coefficients in a range from 380 to 720 nm with a step of 10 nm. The basis for quantitative measurements by diffuse reflectance spectroscopy of colored samples is given by the Kubelka–Munk equation: $F(R)=(1-R)^2/(2R)=2.3\varepsilon c/s$, where $F(R)$ is the Kubelka–Munk function, R is the diffuse reflectance, ε is the molar absorptivity of the sorbate, c is the concentration and s is the scattering coefficient of the sample surface.

The procedure for the measurement of diffuse reflectance spectra is reduced to measuring the diffuse reflectance coefficient

R for a certain wavelength and the calculation of $F(R)$ function for each wavelength λ_i. In some cases the values $\Delta F = F_2 - F_1$ (where F_1 and F_2 are Kubelka–Munk functions of unloaded and loaded PUF, respectively) were calculated.

The spectra and absorbance of solutions were registered on an SF-103 spectrophotometer (Akvilon, Russia); the values of pH were controlled by an Expert 001 potentiometer. HPLC analysis was performed with a "Tsvet Yauza" liquid chromatographic system (NPO Khimavtomatika, Russia). A Luna 5u C18(2) column (150×3.0 mm, 5 μm) was used for the separation of flavonoids. The mobile phase consisted of 25% (v/v) acetonitrile, 74.9% (v/v) water, and 0.1% (v/v) H_3PO_4. The detection was performed with a spectrophotometric detector at 255 nm.

2.3. Procedure

The preconcentration was carried out under batch conditions. Test solutions containing a certain amount of flavonoid in 6.25 ml of ethanol, 0.5 ml of 5 M HCl, and water up to a volume of 25 ml were sequentially added to vessels with ground stoppers. A single PUF tablet was placed in each vessel. Air bubbles were removed using a glass rod, and the vessels were shaken mechanically for 60 min. Tablets were removed and dried between sheets of filter paper, and their diffuse reflectance was measured at 380 nm. The solutions after adsorption were analyzed by UV–vis spectrophotometry.

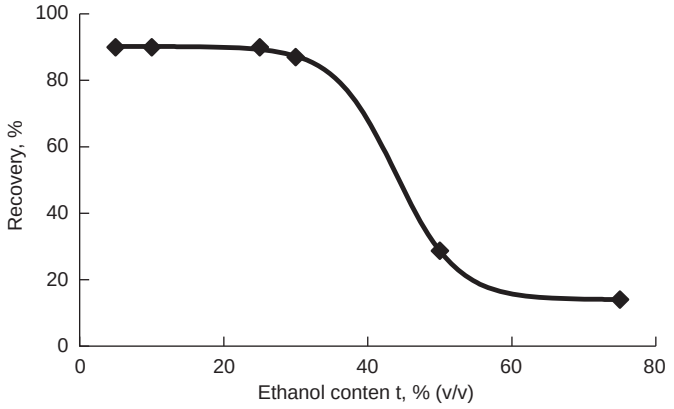

Fig. 2. Effect of ethanol content on the preconcentration of quercetin on PUF. Conditions: V: 25.0 mL; m_{PUF}: 0.020 ± 0.001 g; QU: 5×10^{-5} mol L^{-1}; HCl: 0.1 mol L^{-1}; and contact time, 1 h.

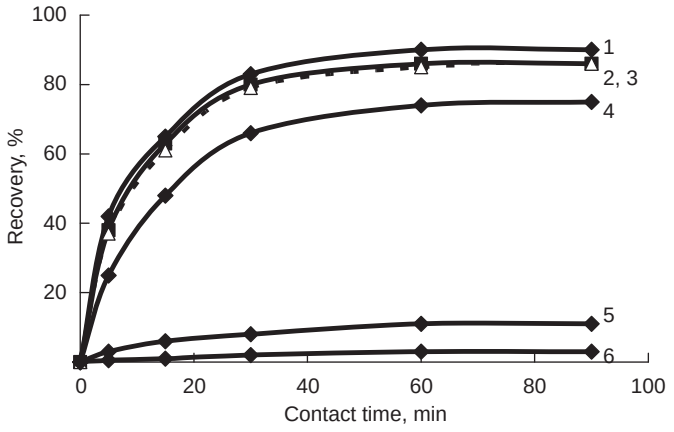

Fig. 3. Effect of contact time on the preconcentration of quercetin (1), morin (2), chrysin (3), naringenin (4), naringin (5) and rutin (6). Conditions: V: 25.0 mL; m_{PUF}: 0.020 ± 0.001 g; FL: 5×10^{-5} mol L^{-1}; HCl: 0.1 mol L^{-1}; ethanol: 25% (v/v); and contact time, 1 h.

The extraction recoveries (efficiencies) (R, %) and the distribution coefficients (D) were calculated as follows:

$$R,\% = \frac{c_0 - c}{c_0} \cdot 100$$

$$D = \frac{R,\%}{(100 - R,\%)} \cdot \frac{V}{m},$$

where c_0 is the initial molar concentration of the tested compound in solution before the sorption, c is the concentration of the tested compound in solution after the sorption, V is the volume of the solution (mL) and m is the mass of the foam (g).

2.4. Sample pretreatment

The analyzed herb and peel onions were crushed, homogenized, and 0.5 g of each sample was extracted with 40 mL of 50% ethanol in an ultrasonic bath at 60 °C for 1 h. The obtained extracts were filtrated, transferred to 50 mL volumetric flasks, and then ethanol and water (1:1) were added up to 50 mL. The aliquots of extraction solutions, the dietary grape polyphenol concentrate "*Enoant*" and ethanol tincture of hawthorn containing 2.5–50 μg of quercetin and ethanol to final content 6.25 ml were placed in a 25 ml vessel, to which 0.5 ml of 5 M HCl, and then water were added up to a volume of 25 ml. The subsequent procedure was similar to that used for calibration.

3. Results and discussion

3.1. Factors influencing the preconcentration efficiency

In order to increase the recovery of the determined substance, the influence of the concentration of ethanol solution, the contact time for sorption preconcentration and pH, the volume of solution and the amount of PUF were investigated.

At first, by the example of quercetin, different concentrations of ethanol solutions were used to perform sorption experiments in order to choose the proper sorption solution (Fig. 2). The optimum medium for preconcentration was found to be 25% (v/v) ethanol. The solvent with this percentage of ethanol enables the best compromise between solubility of the substance in pure water and sufficient recovery.

Secondly, the variation of the recoveries with contact time was investigated. The results are given in Fig. 3. One can see that the

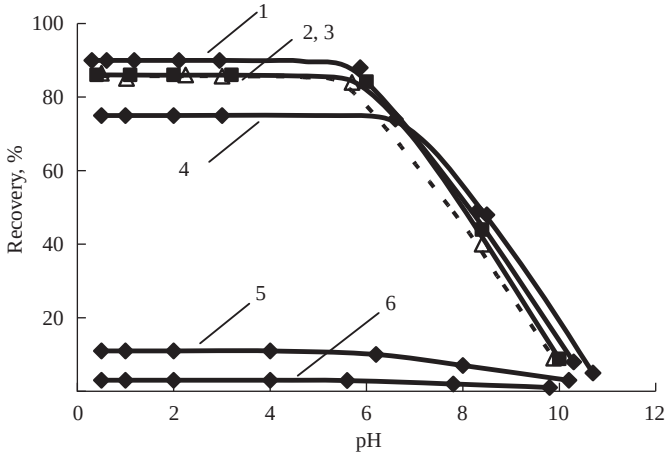

Fig. 4. Effect of pH on the preconcentration of quercetin (1), morin (2), chrysin (3), naringenin (4), naringin (5) and rutin (6). Conditions: V: 25.0 mL; m_{PUF}: 0.020 ± 0.001 g; FL: 5×10^{-5} mol L^{-1}; HCl: 0.1 mol L^{-1}; ethanol: 25% (v/v); and contact time, 1 h.

adsorption equilibrium establishes within 60 min for all the flavonoids investigated.

Flavonoids exist in solution both as neutral and ionized forms. Therefore, pH plays an important role in their sorption on PUF. The effect of pH on the sorption of flavonoids was investigated over a wide range (pH 1–11). As can be seen from Fig. 4, the maximum values of sorption are reached at pH 1–6, the sorption decreases down to zero with an increase in pH. The observed effect gives evidence of the fact that flavonoids are sorbed on PUF in a molecular form.

By the example of quercetin, it was shown that the volume of the solution and the amount of PUF are also key parameters that affect the preconcentration behavior of flavonoids. As one can see from the data given in Table 1, the preconcentration factors g of quercetin which were calculated according to the formula $g=m_1/m_2 \cdot R$, where m_1 and m_2 are the masses of the solution and of the sorbent respectively and R is recovery, increase with the increase in the volume of a solution and with the decrease in the mass of a sorbent.

The recoveries (R, %), distribution coefficients ($\log D$), preconcentration factors and selectivity coefficients relative to quercetin of the flavonoids are summarized in Table 2. The recoveries for quercetin, morin and chrysin determined in the optimized conditions are 86–90% (RSD are 0.9–1.3%, $n=3$) and the recovery for naringenin is 75% (RSD is 0.9%, $n=3$). The presence of glycosidic residue in the molecules of rutin and naringin leads to the sharp decrease in recoveries; these substances are sorbed on PUF with recoveries of 3% and 11% respectively (RSD are 6.7% and 3.7% respectively, $n=3$). High selectivity coefficients for these two substances show an ability to separate from other flavonoids.

Table 1
The recoveries (R, %) and preconcentration factors (g) of quercetin on PUF from different volumes of solutions, $C_{QU}=5\cdot10^{-5}$ mol L^{-1}.

V (mL)	$m_{PUF}=0.020\pm0.001$ g		$m_{PUF}=0.040\pm0.001$ g	
	R, %	g	R, %	g
5	98	245	99	124
15	95	713	96	360
25	89	1113	94	588
50	80	2000	89	1113

Table 2
The recoveries (R, %), distribution coefficients ($\log D$), preconcentration factors (g) and selectivity coefficients (relative to quercetin) of the flavonoids on PUF.

Compound	R, %	$\log D$	g	$\alpha=D_{Querc}/D_{Fl}$
Quercetin	90 ± 3	4.05	1125	1
Naringenin	75 ± 4	3.57	938	3.0
Morin	86 ± 2	3.88	1075	1.5
Chrysin	86 ± 2	3.88	1075	1.5
Rutin	3.0 ± 0.5	1.59	38	288
Naringin	11 ± 1	2.18	138	74

Mean $\pm$ SD ($n=3$, $P=0.95$).

3.2. Spectral characteristics

Flavonoids sorbed on PUF are able to absorb light in the visible range. Diffuse reflectance spectra of flavonoids sorbed on PUF are shown in Fig. 5(a). From the comparison of spectra one can see that in the phase of PUF the most intensively colored are sorbates of quercetin, morin and chrysin. At 380 nm the Kubelka–Munk function has a linear response to the concentrations of quercetin, morin and chrysin in aqueous solutions. As an example the spectra of diffuse reflectance of sorbates of quercetin sorbed on PUF from solutions with different concentrations of quercetin and dependence of the F_{380} value on the concentration of QU are shown in Fig. 5(b).

Table 3
Calibration curve equations of quercetin, morin and chrysin and the related parameters.

Compound	Regression equation	R^2	Linearity range (μg mL^{-1})	LOD (μg mL^{-1})
Quercetin	$\Delta F=14.99\cdot C$	0.996	0.03–2	0.01
Morin	$\Delta F=2.614\cdot C$	0.999	0.2–10	0.07
Chrysin	$\Delta F=0.754\cdot C$	0.998	0.6–25	0.2

Table 4
The results of quercetin determination in artificial samples containing various flavonoids.

Artificial samples[a]	Main additives (μg mL^{-1})	Found of quercetin[b] (μg mL^{-1})	RSD (%, $n=3$)
1	Rutin (25)	0.57 ± 0.08	5.6
2	Naringin (25)	0.53 ± 0.05	3.8
3	Naringenin (25)	0.55 ± 0.06	4.3
4	Rutin (10), Naringin (10), Naringenin (10)	0.56 ± 0.07	5.0
5	Chrysin (0.5)	0.52 ± 0.05	3.9
6	Morin(0.25)	0.54 ± 0.06	4.5

[a] The concentration of quercetin in each artificial sample is 0.5 μg mL^{-1}.
[b] Mean $\pm$ SD ($n=3$, $P=0.95$).

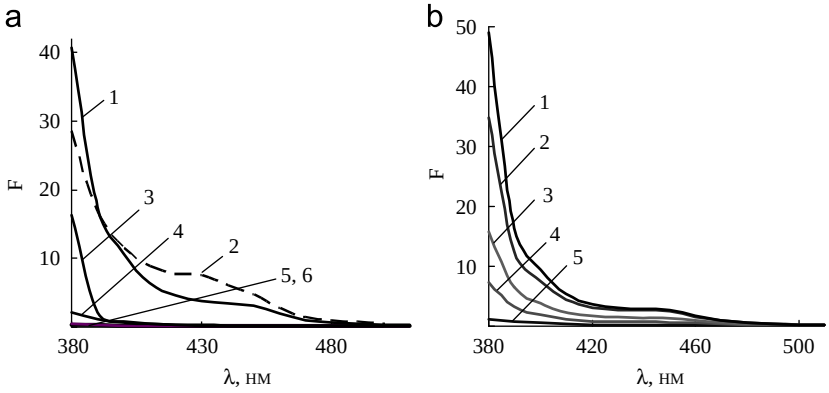

Fig. 5. Diffuse reflectance spectra of flavonoids sorbed on PUF (a) and spectra of quercetin sorbates depending on total quercetin concentration in a solution (b). (a) Quercetin (1), morin (2), chrysin (3), rutin (4), naringenin and naringin (5, 6), $C_{FL}=5\times10^{-5}$ mol L^{-1}; (b) C_{QU}, μg mL^{-1}: 3.0 (1); 2.0 (2); 1.0 (3); 0.5 (4); 0.1 (5).

Table 5
The results of the quercetin determination in different samples.

Sample	Proposed method		HPLC	
	Found of quercetin	RSD (%, $n=3$)	Found of quercetin	RSD (%, $n=3$)
The dietary grape polyphenol concentrate "*Enoant*"	23 ± 5[a]	8.7	18 ± 2[a]	4.5
Peel onions	9 ± 1[b]	4.5	$8.7 \pm 0{,}9$[b]	4.2
Herb (*Monarda didyma L*)t	$0.73 \pm 0{,}07$[b]	3.9	$0.6 \pm 0{,}1$[b]	6.7
Ethanol tincture of hawthorn	14 ± 2[a]	5.7	14 ± 1[a]	2.9

[a] $\mu g\ mL^{-1}$.
[b] $mg\ g^{-1}$.

On the basis of the experimental results, the following conditions for the preconcentration and determination of flavonoids were chosen as optimal for subsequent work: *V*, 25 mL; contact time, 1 h; C_{HCl}, 0.1 mol L^{-1}; concentrations of ethanol, 25% (v/v); and λ_{max}, 380 nm.

3.3. Analytical application

The linear dependence of Kubelka–Munk function of flavonoids in PUF matrix on the concentration of the substances in a solution allows the suggesting of diffuse reflectance determination of some flavonoids. Some features of this method such as the regression equation, correlation coefficient, linearity range and limit of detection were calculated. They are represented in Table 3. It shows that there is a good linear relationship between Kubelka–Munk function and the concentration of the substance in the solution. The limits of detection (LOD) are 0.01, 0.07 and 0.2 $\mu g\ mL^{-1}$ for quercetin, morin and chrysin respectively. The slopes of the calibration curves and the detection limits indicate that the method is the most sensitive to quercetin. This may be used for its determination in the presence of other flavonoids. This supposition was proved by analyzing model mixtures of flavonoids of known composition. The results are represented in Table 4. Since the sorbates of rutin, naringin and naringenin in the phase of PUF are practically not colored the determination of quercetin was not affected by these compounds in the amounts 50 times exceeding those of quercetin as well as by morin and chrysin in the amounts comparable with those of quercetin. Relative standard deviation was not more than 6%.

The applicability of the developed method was demonstrated by the analysis of a variety of real samples, including the dietary grape polyphenol concentrate "*Enoant*", peel onions, herb (*Monarda didyma L*), and an ethanol tincture of hawthorn. The results are summarized in Table 5. The samples were also analyzed by HPLC (see Section 2.2). The results obtained by the proposed method are in accordance with the results obtained by HPLC.

4. Conclusions

In this paper, polyurethane foam was suggested for preconcentration of flavonoids and their direct determination by diffuse reflectance spectroscopy. The optimal conditions for preconcentration are as follows: $V=25$ mL; time$=1$ h; $C_{HCl}=0.1$ mol L^{-1}; and concentration of ethanol$=25\%$ (v/v). It has been stated that Kubelka–Munk function at $\lambda=380$ nm can be used as an analytical response for determination of some flavonoids by diffuse reflectance spectroscopy. The proposed procedure is simple, inexpensive and requires minimal sample pretreatment. The method allows the determining of quercetin in the presence of some other flavonoids.

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