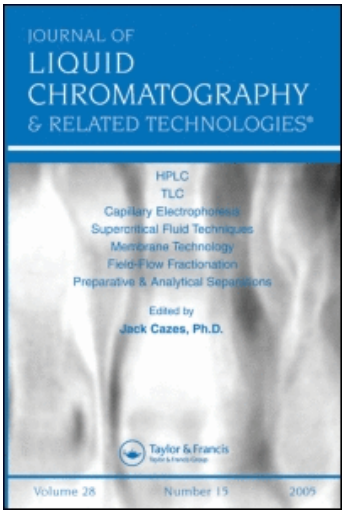

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Supercritical Fluid Extraction of Quercetin and Rutin from *Hyperici* Herba

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ABSTRACT

An analytical-scale method using supercritical carbon dioxide for extraction of flavonoidal compounds from *Hyperici* herba has been developed. Carbon dioxide pressure, temperature, flow, percentage of methanol as organic modifier, and trapping conditions were optimized using an experimental design. Quercetin and rutin were the compounds detected in the HPLC chromatogram of each extract. Recovery and the reproducibility of both compounds were calculated. Under optimized conditions recovery was estimated to be 92% and 76% for quercetin and rutin, respectively, with RSD values equal to 6.4% and 5.2%. Results from the supercritical fluid extraction method were compared with results obtained via a sonication method, using a methanol–water extraction system.

Key Words: *Hyperici* herba; Quercetin; Rutin; Supercritical fluid extraction.

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INTRODUCTION

The use of medicinal plants (herbs) and their extracts as well, has a long history throughout the world, since the ancient times.[1,2] The traditional methods for the extraction of plant materials include steam distillation,[3] and organic solvent extraction using maceration, ultrasonic, and Soxhlet extraction.[3–5] These procedures, however, have distinct drawbacks such as time-consuming and labour-intensive operations, handling a large volume of hazardous solvents, and extended concentration steps, which can result in the loss or degradation of target analytes.[6,7] Consequently, there is increasing interest for alternative extraction technologies consuming less organic solvents, because of the rising solvent acquisition and disposal costs and regulatory restrictions.[7,8]

Supercritical fluids have been shown to exhibit several advantages in the extraction of natural products from plant matrices.[9–11] The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids make them particularly suitable for the extraction of diffusion-controlled matrices such as plant tissues.[12] Moreover, the solvent strength of a supercritical fluid can be easily tuned by simply changing the applied pressure and/or temperature.[6,7,13] Carbon dioxide, the most commonly used supercritical fluid, has the additional advantages of being non-flammable, fairly non-toxic, cost-effective, and easily removed from the extract following decompression. Finally, due to its relatively low critical temperature (31.1°C), thermal sample decomposition is reduced. Pure CO_2, however, is not an appropriate extraction fluid for polar analytes and retentive matrices. In order to enhance the solvating power of CO_2, the addition of a few percent of a modifier solvent is required.[7,13]

Flavonoids are low-molecular-mass compounds found in all vascular plants. They act as antioxidants or as enzyme inhibitors, are involved in photosynthesis and cellular energy transfer processes, and may serve as precursors of toxic substances.[1,12,14,15] Specific flavonoids are known to have pharmacological activity, particularly anti-allergic, anti-inflammatory, anti-viral, or anti-carcinogenic effects. Beside this, flavonoids are believed to be free radical scavengers and metal chelators and inhibitors of lipid peroxidation.[14] *Hypericum perforatum* L. (also known in Anglo-Saxon folk medicine as St. John's Wort) is a herbaceous perennial plant, belonging to the Hypericaceae family, distributed in Europe, Asia, Northern Africa, and naturalized in USA. It is a well-known medicinal plant since antiquity, and was used to heal wounds, remedy kidney troubles, and alleviate nervous disorders, even insanity.[2,16] Today, St. John's Wort is best known for its use in the treatment of mild-to-moderately severe depressive disorders.[17–20] It was one of the top-selling herbal products for 1997.[1]

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A number of studies have reported the employment of the supercritical fluid extraction on flavonoidal compounds from different matrices.[9,10,12,21–24] However, to the best of our knowledge, none of these investigations examined, in detail, the parameters that significantly influence the analytical-scale off-line supercritical fluid extraction, especially on St. John's Wort samples. In this paper, we have focused on optimization of the extraction parameters for rutin and quercetin from a simplified model produced by spiking inert solid material with authentic samples of these compounds. Some previous studies[11,21–24] suggested that the most influential variables for flavonoid efficient recoveries were: supercritical fluid density (pressure), extraction temperature, nature of organic modifier, and its percentage, extraction time, and fluid supercritical flow. Obtained parameters from the optimization procedure were implemented on the plant material. Identification and determination of quercetin and rutin in supercritical fluid extracts was performed by reverse phase HPLC with a diode array detection (DAD) system. Since the age of the raw material can significantly modify the flavonoidal composition, our goal was to develop a method to extract quercetin and rutin from *Hyperici* herba, not to quantify them in this particular plant sample.

EXPERIMENTAL

Chemicals

HPLC grade methanol, acetonitrile, and purified water were supplied by Merck (Darmstadt, Germany). Authentic samples of rutin hydrate (with purity over 98%) and quercetin dihydrate purchased from Sigma (USA) were used for optimization of supercritical fluid extraction conditions, and for their HPLC-DAD qualitative and quantitative determination in obtained extracts. Stock solutions of rutin and quercetin ($1000\,\mu g\,mL^{-1}$) were prepared in methanol and diluted to desired concentrations just prior to use. The solutions were kept in a 4°C refrigerator and were stable approximately for 1 month.

Plant Materials

Hyperici herba samples were supplied by commercial sources. Prior to analysis, the whole herba (grains, leaves, and flowers) was ground and passed through sieves with different pore sizes. For the presented investigations, ground herba with particle diameter 0.300–0.750 mm was used. The moisture content was determined with a Sartorius MA 40 instrument and it was found to be 6.4%.

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Supercritical Fluid Extraction

Supercritical fluid extractions reported here were carried out on a Hewlett Packard 7680T instrument. Extractions were done in 7 mL extraction tubes sealed with caps, while ODS packing was used as a trapping material. Extracted analytes were recovered from the trap by using methanol as eluting solvent. The volume of the eluent was 1 mL, and it passed through the trap at a rate of 0.5 mL min^{-1}. As a supercritical fluid carbon dioxide produced by TGS, Skopje was employed. Solutions of authentic standards for optimization of the extraction conditions were adsorbed on glass wool, produced by Merck, Darmstadt, placed into the extraction tube as an inert solid carrier. By changing the values of the parameters that significantly influence the SFE efficiency like: pressure, temperature, duration of the static and dynamic extraction time, volume of the fluid modifier, trap temperature, and the fluid flow through the system, we have searched for the optimal conditions at which the best extraction efficiency can be obtained. The accuracy and reproducibility of the extraction method was proven by the method of standard addition, by fortifying the sample of *Hyperici* herba with standards of quercetin and rutin at the beginning of the extraction procedure.

Comparative Extraction Method

For comparison of the obtained results from the optimized SFE method as a second extraction method, the one proposed by Dias et al.[4] was employed. Thus, from the described extraction procedure in this paper, we have accepted the methanol/water proportion that they have found to be the most appropriate. Five hundred milligrams from ground herba was extracted, in triplicate, with 3 mL of methanol/water mixture 80 : 20 (v/v) by sonication at room temperature for 30 min. Extracts were first filtrated through Whatman filter paper (black label) and afterwards they were filtrated through 0.2 μm membrane filter discs (Sartorius) into autosampler vials. These extracts were directly injected into the chromatographic system.

HPLC-Diode Array Detection Analysis

Extracts were analyzed with HPLC-DAD on a Perkin Elmer system equipped with quaternary LC pump series 200, autosampler series 200, Diode Array Detector 235C within wavelength range from 190 to 365 nm.

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Supelcosil LC 18 analytical column with 250 mm length, 4.6 mm internal diameter, 5 μm particle size, maintained at 30°C in a thermostat. For data collecting and processing, Perkin Elmer Turbochrom software was used. Chromatograms were recorded at 270 and 330 nm, according to the HPLC method proposed by Brolis et al.,[5] while UV spectra were collected during the whole run. For the mobile phase, acidified water with formic acid (pH 2.25–2.30) was used as eluent A, acetonitrile as eluent B, and methanol as eluent C. The pump program was performed in four steps, whereas starting with 100% of A, 0% of B, and 0% of C, in 20 min a mobile phase consisted of 80% A, 15% B, and 5% C was obtained. In the next step, in 10 min the composition of the mobile phase was changed to 10% A, 70% B, and 20% C. The total separation was finished in 30 min and the establishing of the initial conditions was achieved in 5 min, with additional 10 min for column equilibration prior to the next run. Injection volume was 25 μL, and the flow velocity was 0.7 mL min^{-1}. Analytes were identified by comparing their retention times, and UV–Vis spectra with those of the authentic compounds. The accuracy of the HPLC method was checked with the procedure of a standard addition, by spiking the obtained SF extracts of *H. perforatum* prior to the instrument analysis.

RESULTS AND DISCUSSION

Calibration Procedure for HPLC

For both quercetin and rutin, quantification was done with establishing calibration curves by plotting the area of peaks obtained at 270 and 330 nm against different concentrations of examined compounds, within the concentration range from 1 to 100 μg mL^{-1} (expressed in a mass of rutin and quercetin injected into the column from 25 to 2500 ng). Limits of detection (LOD) and quantification (LOQ) were calculated as 3 times and 10 times standard deviation of the calibration curve, respectively, constructed for the low concentration range from 1 to 40 μg mL^{-1} (25–1000 ng injected into the column) divided with the slope.[25] For quercetin, the calculated LOD for 270 nm was 2.9 μg mL^{-1} and for 330 nm was 4.2 μg mL^{-1}, while the LOQ values were found to be 9.8 and 13.8 μg mL^{-1}. Respective LOD values for rutin were 3.5 and 3.4 μg mL^{-1}, LOQs were 11.5 and 11.4 μg mL^{-1}.

For quantification purposes only, calibration at 270 nm for both rutin and quercetin will be used, since the values of LOD and LOQ for quercetin are lower at this wavelength, although 270 nm is not the most specific for HPLC analysis of flavonoidal compounds. However, we decided to record the chromatograms at this wavelength as well. The reason for this was our

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intention to develop and evaluate HPLC methods, which will be suitable for determination of other constituents of *Hyperici* herba[5] during our further investigations.

Optimization of SFE Conditions

In SFE, pressure and temperature are the two most important instrumental parameters. Together they define the density of the supercritical CO_2 and affect the solubility of analytes, which in turn affects SFE yields. Moreover, temperature and pressure have opposite effects on the density of the supercritical fluid. Variations in the combination of pressures, temperatures, and modifiers distinctly affect supercritical fluid solvent power. When a supercritical fluid modifier is used, depending on its percentage (v/v) content in the fluid, a decrease in the density values occurs unlike pure supercritical fluid. Some investigations[26] suggest that more significant density decreasing is exhibited when higher temperatures and lower pressures are applied. The true density values of fluid mixture at certain temperatures and pressures might be either calculated by some suggested methods, or measured by employing appropriate devices.[26] Since this needs additional effort, we concentrated on controlling the pressure and temperature values. So, the first step in our optimization study was to evaluate the most appropriate temperature and pressure for effective SFE of quercetin and rutin.

The suitable supercritical fluid not only must be capable of solvating target analytes but also must be able to efficiently interact with the analyte-matrix complex to promote rapid partitioning of the analyte into the bulk supercritical fluid.[27] Using pure CO_2, only small quantities of rutin and quercetin were extracted (recoveries were under 1% for severe conditions), so in the further investigations, only extractions with addition of a CO_2 modifier were performed. This was not an unexpected result, knowing the rutin and quercetin properties.[28] The performances of the supercritical fluid extractor which was available to us, dictated the use of a static modifier addition. Having in mind the earlier investigations from the other authors,[9,22] we decided that methanol would be a suitable modifier for our analytes and matrix as well.

According to the structure and chemical properties of the flavonoidal compounds,[10] they are moderately to highly polar, thus they require long extraction times and severe extraction conditions (P, T), as well as modifier addition. To determine the optimal conditions for extraction of quercetin and rutin with modified supercritical CO_2, their solubility (expressed through the recoveries) as a function of the applied pressure at three different temperatures were studied. The obtained recoveries, as well as their RSDs at 40°C, 50°C, and 60°C, are presented in Table 1.

Table 1. Recoveries of quercetin (Q) and rutin (R) from spiked glasswool at 40°C, 50°C, and 60°C.

Recoveries (RSD)(%)								
40°C			50°C			60°C		
P (bar)	Q	R	*P* (bar)	Q	R	*P* (bar)	Q	R
134	14.16	11.82	105	8.74	11.53	93	24.73	15.30
	(4.71)	(3.50)		(8.30)	(5.77)		(2.35)	(4.84)
164	21.42	11.77	151	12.57	9.78	142	19.91	15.20
	(4.64)	(4.67)		(5.09)	(4.89)		(3.59)	(4.65)
190	26.36	14.61	197	26.68	14.98	187	20.06	18.54
	(2.63)	(4.29)		(2.42)	(3.45)		(6.69)	(3.29)
211	30.46	21.58	223	34.45	18.82	226	21.51	26.05
	(4.61)	(3.96)		(2.51)	(3.04)		(3.08)	(4.03)
245	37.76	33.34	257	42.91	35.77	264	28.27	36.79
	(1.17)	(4.66)		(2.93)	(1.80)		(4.15)	(3.90)
281	46.38	58.12	299	56.87	45.67	300	35.30	44.59
	(2.02)	(2.93)		(1.39)	(3.59)		(3.05)	(5.39)
338	70.35	62.08	332	96.74	74.40	345	77.92	58.30
	(1.43)	(4.77)		(2.19)	(4.20)		(3.47)	(3.55)
383	56.41	27.68	370	77.00	59.49	380	54.92	48.53
	(3.02)	(1.90)		(2.06)	(5.35)		(6.54)	(5.55)

All extractions were done in three replicates for $\gamma = 50\,\mu g\,mL^{-1}$ in the final extract. Static extraction time was 30 and dynamic extraction time was 60 min. The volume of the applied methanol as a modifier was 15%, counted on the extraction chamber volume. The best recoveries are obtained at 50°C for both compounds (96% for quercetin and 74% for rutin) when the pressure value was 332 bar. The high pressure values at which the best efficiencies are obtained, confirm the assumption that for quercetin and rutin extraction severe conditions should be applied. The recoveries improvement, when 50°C was employed as an extraction temperature, might be understood as increasing the supercritical fluid diffusibility into the matrix, and hence, increased the extraction rate. But the recovery drop in the case when extraction was performed at 60°C was unexplainable to us, although a suspicion for possible partial analyte degradation could not be excluded.

We should emphasize that when a static mode of a modifier addition is employed its, is important to bring the sample into contact with the modifier over a enough long interval (as long as the imbibition time). It means that the

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duration of the equilibration time is a critical parameter that significantly influences the efficiency of extraction when a static modifier addition is used. We have optimized the static extraction time by changing its duration from 10 to 40 min. On Fig. 1 the dependence of the recovery of extracted quercetin and rutin from the equilibration time is shown. As it can be noticed, their best recoveries were gained when the equilibration time reached 30 min, and the further increasing of the time had no significant positive influence on the efficiency.

The time during which extraction is allowed to proceed is very influential on achieving adequate efficiency, but it also has a strong effect on selectivity. The supercritical fluid solvent power and the variability of analyte–matrix interactions result in widely variable temporal limits for SFE.[13] The recovery of the analytes from the matrix affected by the dynamic extraction time was examined in the interval from 10 to 60 min, while the other extraction parameters were with constant values. On Fig. 2, a correlation between the dynamic time extraction and analytes recovery is presented. The best efficiency was obtained after a 55 min extraction, although when herba extracts were performed the time was extended for an additional 5 min.

Our first choice for a CO_2 modifier was methanol, which has been proven as a suitable modifier in the SF extraction of flavonoids and other polyphenolic compounds from plant materials.[9,22] The influence of the methanol amount added in the extraction thimble, on the recovery of rutin and quercetin, is presented in Fig. 3. The best recoveries were exhibited when using 10 and 15% (v/v) methanol, counted on thimble volume. However, bigger methanol percentages caused the recoveries to decrease. A methanol amount of 15% was

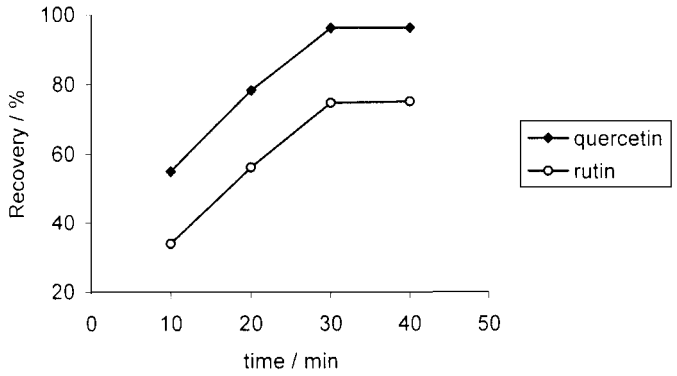

Figure 1. Plot of extraction efficiency vs. static extraction time of rutin and quercetin extracted at 332 bar and 50°C; 60 min dynamic extraction with addition of 15% (v/v) methanol.

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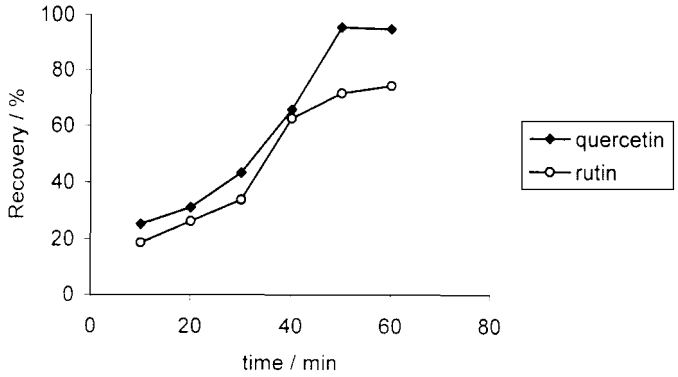

Figure 2. Plot of extraction efficiency vs. dynamic extraction time of rutin and quercetin extracted at 332 bar and 50°C; 30 min static extraction with addition of 15% (v/v) methanol.

selected for further experiments. The main disadvantage of the static addition mode is that, as supercritical fluid starts to circulate through the sample, the modifier is swept from the extraction cell, so the matrix is brought out of contact with the modified CO_2. On the other hand the gradually decreasing of the modifier amount in the fluid, disables the possibility for the analyte rinsing through the analytical trap during the extraction step, so higher amounts of CO_2 than 2–5%, as is recommended for dynamic modifier addition,[6] can be employed.

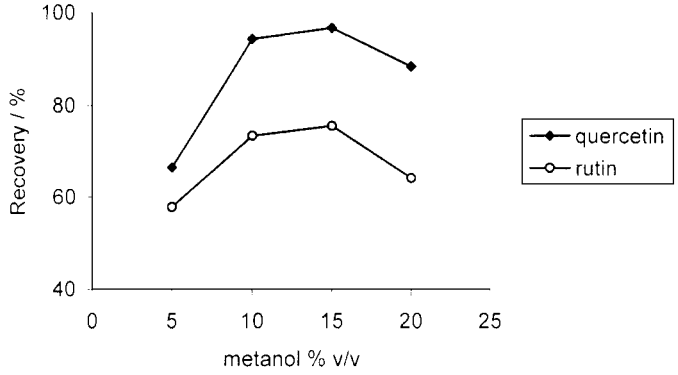

Figure 3. Plot of extraction efficiency vs. methanol amount of rutin and quercetin extracted at 332 bar and 50°C; 30 min static and 60 min dynamic extraction.

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Collection of rutin and quercetin was observed on ODS packing. To obtain the best trapping conditions for rutin and quercetin, extractions from spiked glasswool as inert carrier were performed by changing the trap temperature from 10°C to 60°C, at temperature of 50°C and pressure of 332 bar. The recoveries of rutin and quercetin are given in Fig. 4. Since the analytes are non-volatile, recoveries of the substances were improved by increasing the trap temperature, and the best recovery was performed when the trap temperature was 50°C. This is in good agreement with the previous conclusions for non-volatile compounds with high boiling points (over 200°C).[6]

The trap temperature during the collecting process should be high enough to prevent modifier condensation and analytes rinsing, resulting in an inefficient extraction. When methanol is used as a CO_2 modifier, a temperature from 40°C to 60°C should be convenient for our types of analytes, and higher trap temperatures may cause partial analytes decomposition. Moreover, the optimal trapping temperature also depends on the percentage of the modifier used. When concentration greater than 2% is used, trap temperatures of at least 40–50°C are required to obtain efficient trapping.[6]

The flow rate of the supercritical fluid seems to be another parameter that influences the extraction efficiency. Higher flow rates provide the sample with a larger quantity of fresh extraction fluid, while lower rates require less extraction fluid and often simplify the collection of the extracted analytes.[29] On Fig. 5, the extraction rates, expressed through the recoveries of quercetin and rutin at four different flow rates, 0.5, 1.0, 1.5, and 2.0 mL min^{-1} can be observed. The increase of the flow rate from 0.5 to 1.0 has no significant influence on the extraction efficiency. Hence, the further increasing of the flow

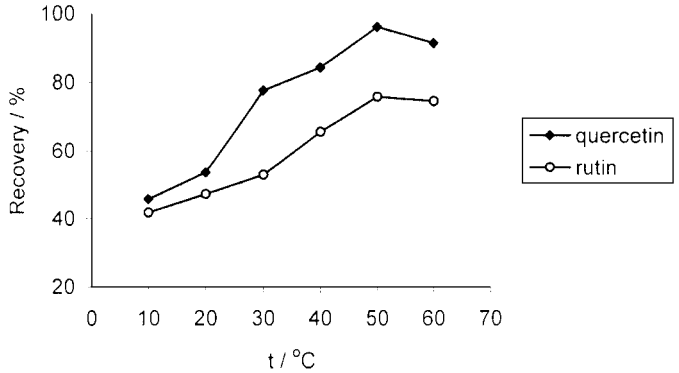

Figure 4. Plot of extraction efficiency vs. trap temperature time of rutin and quercetin extracted at 332 bar and 50°C; 30 min static and 60 min dynamic extraction with addition of 15% (v/v) methanol.

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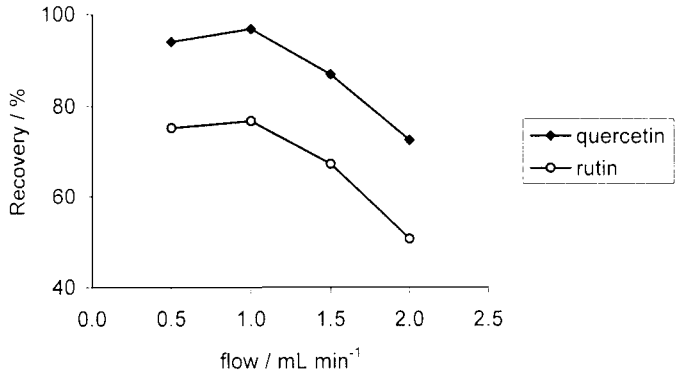

Figure 5. Plot of extraction efficiency vs. supercritical fluid flow of rutin and quercetin extracted at 332 bar and 50°C; 30 min static and 60 min dynamic extraction with addition of 15% (v/v) methanol.

rate from 1.0 to 2.0 mL min^{-1} drops the extraction efficiency approximately 20%. This is very probably due to the difficulty in efficiently collecting quercetin and rutin from the analytical trap.

SFE of *Hypericum perforatum*

Prior to the extraction, *Hyperici* herba was ground and 500 mg of the sample was measured into the extraction chamber. Fifteen percentage of methanol (v/v) was added to the sample, and the SF extraction was performed at the parameters that occur to be the most appropriate during the optimization procedure: pressure of 332 bar and temperature of 50°C; static extraction time was 30 min, and dynamic extraction was 60 min; trap temperature was 50°C for extraction, and 30°C for the rinse step; the flow rate of a supercritical CO_2 was 1.0 mL min^{-1}. After the extraction step was completed, analytes were recovered in 1.0 mL methanol, which passed through the trap at a rate of 0.5 mL min^{-1}. Each extraction was done in replicates of three. In Fig. 6, a chromatogram from the obtained sf extract at 270 nm is presented.

In the lack of a referent standard material, the accuracy of the extraction procedure should be checked by the method of standard addition. According to some authors, the acceptance of the standard addition method as the only validation parameter may be questionable because native and spiked compounds can show different affinities toward matrix sites.[12] Native compounds usually are distributed throughout the plant matrices, but the spiked compound does not necessarily mean high recovery of the native

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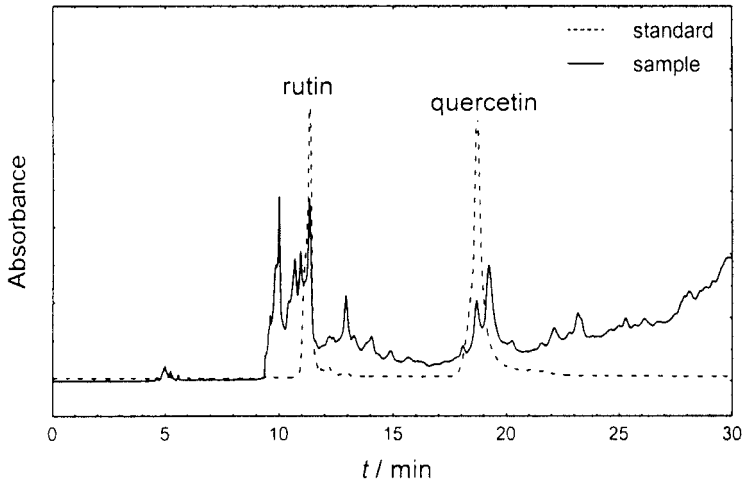

Figure 6. Chromatograms of standard solutions of rutin and quercetin and SF extract from *Hyperici* herba recorded at 270 nm.

compounds. Thus, besides testing the SFE accuracy through the recoveries obtained from the standard addition methods, a comparison of the SFE with other extraction methods may be needed.[30]

As a second extraction method, we have chosen to employ the method proposed by Dias et al.[4] with the difference that we shortened the extraction time by accelerating the extraction in an ultrasonic bath, instead of 24 hour extraction at room temperature. In Table 2, the results from the two extraction methods for quercetin and rutin from *Hyperici* herba are compared.

From the presented results, it can be concluded that the supercritical fluid extraction procedure recovered higher amounts of quercetin than the extraction performed by sonication. On the other hand, the extracted amount of rutin with sonication was almost three times higher than the one obtained by SF extraction. These phenomena could be explained through the chemical properties of those compounds. The molecule of rutin has a much bigger molecular weight, and also, it is more polar than the molecule of quercetin. Consequently, the solubility of rutin in SC CO_2 is smaller, which was also concluded when SF extraction parameters were optimized by determining the recoveries of the authentic samples from spiked glasswool (the maximum recovery for rutin was 74%). It is evident, that SFE could be employed as a satisfactory efficient extraction technique for moderately polar aglycone flavonoidal compounds. Almost the same conclusions were presented in the published papers from other authors,[9,10] although the investigations were

Table 2. Comparison results from the determination of quercetin and rutin in obtained extracts from 500 mg *Hyperici* herba with particles diameter 0.300–0.750 mm.

	Employed method			
	Supercritical fluid extraction		Sonication	
Compound	γ ($\mu g\,mL^{-1}$)	RSD (%)	γ ($\mu g\,mL^{-1}$)	RSD (%)
Quercetin	71.604	7.86	59.931[a]	4.92
Rutin	76.618	4.59	200.676[a]	1.27

[a]Values are normalized on 1 mL of extract.

carried out on different plant representatives. In Fig. 7, the chromatograms of the obtained extract by the sonication method recorded at 270 nm are presented. For testing the accuracy of the whole SF extraction procedure, the standard addition method was applied. Two hundred milligrams of ground herba with particle diameter 0.150–0.300 mm were spiked with authentic samples of quercetin and rutin at three concentration levels: 10, 20, and 50 $\mu g\,mL^{-1}$.

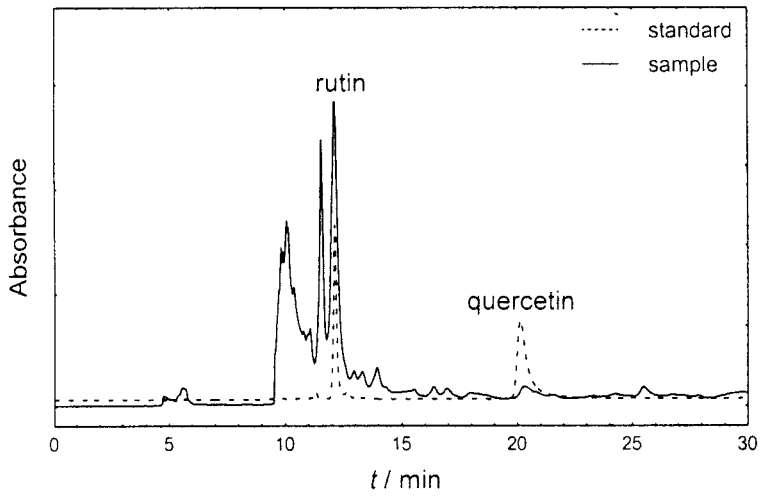

Figure 7. Chromatograms of standard solutions of rutin and quercetin and ultrasonic extract from *Hyperici* herba recorded at 270 nm.

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From the presented results of the standard addition method in Tables 3 and 4 for checking the HPLC method and SF extraction procedure accuracy, it can be concluded that the presented HPLC method exhibited satisfactory high values for the recovery (98.45–101.69% and 99.04–102.43% for quercetin and rutin, respectively). Regarding the SF extraction method, obtained recovery values from *Hyperici* herba matrix were approximately similar (for rutin) and slightly lower (for quercetin) than the observed recoveries when inert solid material was spiked.

CONCLUSIONS

It was shown that when pure authentic samples of quercetin and rutin were extracted from inert solid material, the best recoveries were obtained when temperature of 50°C, pressure of 332 bar were employed, followed by static addition of 15% methanol (v/v) as CO_2 modifier. Under these conditions, the obtained recovery for quercetin was rather satisfactory (around 96%), while it was less effective for rutin (74%). This conclusion was confirmed by checking the extraction procedure accuracy with the method of standard addition, and by comparing the obtained SFE extracts with extracts gained by the extraction with an ultrasonic bath. Regarding the moderately

Table 3. Results from the quercetin standard addition methods for checking the accuracy of the HPLC method and the extraction procedure for the sample of *H. perforatum*.

Measured[c] γ ($\mu g\,mL^{-1}$)	Added γ ($\mu g\,mL^{-1}$)	Calculated[c] γ ($\mu g\,mL^{-1}$)	Recovery (%)[c]	RSD (%)
		HPLC Method[a]		
33.900	—	—	—	—
44.262	10.0	43.900	100.82	2.31
53.067	20.0	53.900	98.45	1.91
85.319	50.0	83.900	101.69	2.16
		Extraction procedure[b]		
60.019	—	—	—	—
60.612	10.0	70.019	86.57	5.05
73.257	20.0	80.019	91.55	6.33
93.073	50.0	110.019	84.60	2.60

[a]From sample with particles size 0.300–0.750.
[b]From sample with particles size 0.150–0.300.
[c]Main values of three replicates.

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Table 4. Results from the rutin standard addition method for checking the accuracy of the HPLC method and the extraction procedure for the sample of *H. perforatum*.

Measured[c] γ ($\mu g\,mL^{-1}$)	Added γ ($\mu g\,mL^{-1}$)	Calculated[c] γ ($\mu g\,mL^{-1}$)	Recovery (%)[c]	RSD (%)
		HPLC Method[a]		
35.079	—	—	—	—
44.648	10.0	45.079	99.04	2.92
55.136	20.0	55.079	100.10	3.13
87.149	50.0	85.079	102.43	1.15
		Extraction procedure[b]		
43.251	—	—	—	—
41.067	10.0	53.251	77.12	2.70
49.839	20.0	63.251	78.80	3.90
72.925	50.0	93.251	78.20	3.32

[a]From sample with particles size 0.300–0.750.
[b]From sample with particles size 0.150–0.300.

polar quercetin, SFE provides a rapid and satisfactory quantitative method for its isolation. However, the disadvantage of supercritical CO_2 for extraction of the more polar rutin with a higher molecule weight is also apparent.

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