Application of the MSPD Technique for the HPLC Analysis of Rutin in *Sambucus nigra* L.: The Linear Correlation of the Matrix Solid-Phase Dispersion Process

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Abstract

This paper presents possibilities of estimating the true value of an analyte concentration in a plant material by means of the matrix solid-phase dispersion (MSPD) based on the law of the partition process and on the properly adapted equations associated with this process. This idea has been developed for analysis of rutin amount in *Sambucus nigra* L. (elderberry). The effects of experimental variables, such as the mass ratio of sorbent to plant matrix and buffer pH, have been studied. Rutin amounts estimated using MSPD were verified with the amounts obtained by employing pressurized liquid extraction (PLE). The extracts were analyzed by high-performance liquid chromatography (HPLC). To obtain the true value of rutin concentration in elderberry, the MSPD process should be carried out in the conditions for which the dependence between the reciprocal of the analyte yield and sorbent mass/plant mass ratio is linear. Rutin yield estimated by MSPD in such conditions equal 2.90% w/w and is almost the same as the amount obtained by PLE (2.88% w/w). The results show that PLE, which recently has been more and more frequently used in the analysis of herb material and which requires the application of expensive PLE equipment, can be replaced by the relatively cheap and effective MSPD process.

Introduction

In general, a broad spectrum of extraction techniques (Soxhlet extraction, percolation, maceration, digestion, extraction under reflux, steam distillation, etc.) are currently being used as samples preparation procedures (1–4). Exhaustive extraction in the Soxhlet apparatus is employed most frequently for this purpose. Although this method is relatively simple, it suffers from disadvantages such as a long extraction time, relatively high solvent consumption, and often an unsatisfactory reproducibility (5). Recently, an innovative sample preparation technique, pressurized liquid extraction (PLE), has been more and more often applied (6–17). The growing popularity of this technique results mainly from the possibility of eliminating the drawbacks mentioned previously. High pressure and high temperatures are used in PLE. High pressure allows the extraction cell to be filled faster, which helps to force liquid into the pores of matrices and to use an extrahent at a temperature above its normal boiling point. The increase of extraction temperature results in better solubility, higher diffusion rates, and mass transfer of extracted compounds. High extraction yields combined with easy operation and automation of this technique resulted in the employment of PLE in various analytical areas, including environmental studies, pharmaceuticals and food stuffs (6–13). An interesting and important application of PLE is extraction of chemical constituents from plant materials (14–17).

For an efficient extraction to occur, the material submitted to the extraction procedure should be carefully ground to increase the contact of solvent with the target analyte. Matrix solid-phase dispersion (MSPD) is a sample preparation procedure involving simultaneous disruption and extraction of various solid, semi-solid, and/or highly viscous biological samples (18–23). The application of MSPD is based on blending of a sample with an abrasive solid support material (C_{18} derivatized silica is used most frequently), packing a cartridge with the resulting slurry, and eluting it with a solvent. The bound organic phase, created during the process, acts as a solvent that dissolves and disperses the sample components into the bound phase. So far, MSPD has been optimized by fitting an appropriate sorbent type and finding the sample to sorbent mass ratio and/or pH etc., in order to obtain the maximum yield of an analyte (18–22). The MSPD process possesses a chromatographic characteristic that may selectively elute a single compound or several classes of compounds from the sample. Although MSPD has been applied to many different matrix types (animal tissues, fruits, vegetables, herbs, foods, soils) and to an equally wide range of target analytes (drugs, pollutants, herbicides, pesticides, and other compounds), it is not so popular as the extraction techniques and is still treated only as an auxiliary procedure. It probably is connected with the fact that MSPD is laborious.

The present paper answers the question whether MSPD can be described as a partition process using equations analogous to those for the liquid–liquid extraction process.

The following discussion concerns the analysis of rutin in *Sambucus nigra* L. using MSPD as a sample preparation method. Rutin (rutoside, 3,3',4,5,7'-pentahidroxyflavone-3-rhamnoglucoside), belonging to a large family of secondary plant metabolites (flavonoids), is very helpful for the correct func-

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tioning of the human organism (27–29), and green plants are the only source of the compound (30). Its analysis in plant matrices is of prime importance. So far, high-performance liquid chromatography (HPLC) following proper sample preparation procedures has been the main method used (31,32). The results estimated by MSPD are verified in relation to the analogous data obtained by means of PLE.

Experimental

Matrix solid-phase dispersion procedure

An adequate weight of ground flowers of *Sambucus nigra* L. (Herbapol-Lublin, Lublin, Poland) and 800 mg of the C_{18} sorbent (Supelclean LC-18, Supelco Park, Bellefonte, PA) were weighed precisely and mixed in a glass mortar in proper mass ratio. The physicochemical parameters of the used sorbent were as follows: particle size d_p = 45 µm, pore volume V_p = 0.8 cm^3/g, average pore diameter D = 6 nm, specific surface area S = 475 m^2/g. After the addition of water or buffer solution (1.5–2.5 mL), the whole mixture was ground with a glass pestle until a homogeneous pulp was obtained. The sides of the mortar and the pestle were scraped occasionally with a spatula to ensure the best possible homogenization. After homogenization, the blend was quantitatively transferred into a 5-mL syringe barrel containing a filter paper at the bottom. The packing material was covered with another circle of filter paper and compressed using the syringe plunger. Portions of the methanol–water mixture (80–20% v/v) were then added to the column, and the sample was allowed to elute dropwise by applying a slight vacuum. 25 mL samples were collected. Separate experiments proved that the rutin concentration in the 20th mL of the eluate is below the limit of detection. Each MSPD procedure was repeated five times.

The following buffers were used in the MSPD procedures: acetate buffers in pH range 3.6–5.5; citrate buffers in pH range 2.2–7.5; phosphate buffers in pH range 2.0–3.2 and 5.6–8.0. The range of mass ratios of the sorbent to the plant matrix in these procedures were from 4:1 to 160:1. Most of the experiments was carried out at 4:1, 10:1, 20:1, and 40:1 sorbent to plant matrix mass ratio.

Pressurized liquid extraction

PLE was performed with a Dionex ASE-200 (Dionex Corp., Sunnyvale, CA). The system consists of a solvent module, pump, thermostatted extraction cell containing the material to be extracted, an electrovalve, and an extract collection vessel. To begin extraction, a portion of plant material (0.4 g or 1.0 g) was mixed with Brazilian quarc (glass sand) and placed into a 22-mL stainless steel extraction cell. The employments of a dispersion agent, such as Brazilian quarc, is recommended in order to reduce the volume of a solvent used for the extraction (33). After loading the cell into the oven, a solvent was pumped into the cell. When the cell was full of the extraction solvent, the cell was heated at the preset extraction temperature and pressurized for a fixed time to ensure that the sample reached thermal equilibrium. During the heating step, the solvent was pumped in and out of the cell to maintain the pressure. The sample was extracted in the following conditions: extrahent, methanol–water mixture (80:20% v/v); extraction temperature, 100°C; pressure, 100 bar; static extraction time, 10 min.

The previous conditions were established in a separate investigation as optimal for the PLE of rutin from *Sambucus nigra* L. (34). After the extraction, the extract was allowed to flow into the collection vessel, and the sample was rinsed with a fresh portion of the solvent (60% of the cell volume). Finally, the sample was purged for 120 s by applying pressurized nitrogen (150 psi). The collected extract (25–31 mL) was transferred into 50-mL laboratory flask and filled up to its volume with pure extrahent. Two types of pressurized liquid extractions were performed: one cycle extraction and multiple extraction of the same sample. Each extraction procedure was repeated five times.

HPLC analysis

Rutin concentrations in the obtained extracts were determined by means of HPLC. Measurements were carried out using a Dionex liquid chromatograph DX600 (Dionex Corp., Sunnyvale, CA) consisting of a chromatography enclosure LC20 with a PEEK automated Rheodyne injection valve having a 25-µL sample loop, a gradient pump GP50, a UV–VIS detector AD25, and a photodiode array detector PDA100. During the course of each run, the absorbance spectra from PDA100 (in the range 190–750 nm) were collected continuously.

Chromatographic separations were performed applying a Prodigy ODS-2 column (250 × 4.6 mm i.d., 5 µm, Phenomenex, Torrance, CA) with a guard column of the same firm. The mobile phase composition was optimized to receive a rutin peak separated from other components of the examined extracts. A mixture composed of CH_3CN (HPLC-grade, POCh, Gliwice, Poland) and MilliQ deionized water (Millipore, Bedford, MA) containing 5% glacial acetic acid (analytical-reagent grade, POCh, Gliwice, Poland) (25:75%, v/v) was employed as mobile phase (flow rate 1 mL/min) in all the chromatographic separations. Each extract was HPLC-analyzed three times.

The identification of the rutin peak was carried out by comparing the retention time of the peak and its UV–Vis spectra with that of the rutin standard (E. Merck, Darmstadt, Germany). The rutin concentrations in the resulting extracts were calculated from calibration curve obtained using five standard solutions of rutin in the concentration

Table I. Equation Describing 1/E(%) vs. m_p/m_s Linear Dependences of MSPD Process Obtained in Given Conditions

No	Buffer type used in the MSPD mixing process, pH	Range of (m_s/m_p)	Equation of ($1/E_{(\%)}$) vs. (m_p/m_s)	Correl. Coeff. R^2	Total rutin amount (E(%)total) in [% w/w]
1	Acetate, pH 3.6	4–40	$0.298 \cdot \frac{m_p}{m_s} + 0.3448$	0.9995	2.90
2	Acetate, pH 4.5	26.7–160	$2.208 \cdot \frac{m_p}{m_s} + 0.3352$	0.9901	2.97
3	Phosphate, pH 2.0	26.7–80	$1.697 \cdot \frac{m_p}{m_s} + 0.3501$	0.9999	2.86

range from 0.005 mg/mL to 0.040 mg/mL (y = 611.5143x + 0.0679; R^2 = 0.9999; error of slope estimation = 3.5154; error of intercept estimation = 0.0864). The limit of quantification of rutin (calculated as signal-to-noise ratio equaled 10) was 0.0623 µg/mL.

Results and Discussion

The preliminary investigations of the rutin amount in *Sambucus nigra* L. flowers revealed 0.848 (± 0.011)% w/w of the compound by means of MSPD and 2.041 (± 0.024)% w/w by means of PLE. It should be stressed that the presented results were obtained at the 4:1 ratio of sorbent to plant matrix in MSPD and by one cycle extraction of 1 g plant sample in PLE. The estimated amount of rutin (rutin yield) in the flowers is significantly lower ($p = 1.05 \times 10^{-13}$) when MSPD is employed in the analytical procedure than in the case of the PLE method. The analogous relation in rutin amounts isolated from the leaves of *Ficus carica* using MSPD and solid–liquid extraction process was reported in (19–35). Considering the previously mentioned discrepancy shown in Table I, the question appears whether the sorption capacity of the sorbent employed in MSPD was sufficient in relation to the relatively large rutin amount in the plant matrix. To answer it, another series of experiments was carried out in which different ratios of sorbent to the flowers were used in MSPD. The results of this series are presented in Figure 1, *curve A*. The obtained relationship shows that when mass ratio increases, a greater rutin amount is found in the flowers. However, at the maximum mass ratio used in these experiments (800 mg: 20 mg = 40:1), the amount of rutin established using MSPD was still considerably lower than using one PLE cycle (1.32% w/w using MSPD, see Figure 1, *vs.* 2.041% w/w using PLE).

It is worth mentioning that the shape of *curve A* in Figure 1 is analogous to the shape of relationship described by equation (1), which presents the recovery (fraction) of a substance (E) in the liquid–liquid extraction process as a function of the volume ratio of liquid phases $V = V_o/V_w$ (36–37):

$$E = \frac{K_0 \cdot V}{1 + K_0 \cdot V} \qquad \text{Eq. 1}$$

where K_0 is a partition coefficient of the substance between two phases, and V_0 and V_w are volumes of the organic and water phases, respectively. Transforming the previously mentioned equation into its linear form, the total amount of substance contained in one of the phases of the liquid–liquid extraction system can be calculated:

$$\frac{1}{E} = \frac{1}{K_0 \cdot V} + 1 \qquad \text{Eq. 2}$$

$$\frac{1}{E_{total}} = \lim_{\frac{V_0}{V_w} \to 0} \frac{V_W}{K_o \cdot V_o} \qquad \text{Equ. 3}$$

For a well matched system, the first step of the applied MSPD process (mixing plant matrix with sorbent at the presence of a liquid) can be considered more or less as a liquid–liquid extraction. If it is true, equation (3) could be transformed to the analogous equation (4), which would help estimate the total rutin amount (%) in the flowers.

$$\frac{1}{E} = \frac{m_p}{K_C \cdot m_8} + C \qquad \text{Eq. 4}$$

where m_p and m_s are the masses of the plant matrix and sorbent in the MSPD process, respectively. K_C implicates a partition coefficient of the substance between the plant matrix and sorbent and density ratio of the phases.

The function $1/E_{(\%)} = f(m_p/m_s)$ corresponding to reported above experiments with different ratios of sorbent to flowers is presented in Figure 1, *curve B*. The run of this curve shows that there is no possibility to find the true ($1/E_{(\%)total}$) value because the dependence is not linear. The non-linearity of the last relationship shows that the sorption capacity of the applied sorbent, even for greater mass ratio of sorbent to plant, is still too low or the system is not well matched and there are other important factors that affect the partition process in MSPD. They should be recognized and stabilized (i.e., the last relationship should be measured in stable pH, at a carefully controlled temperature, or in the range of m_s/m_p ratios greater than 40:1).

It should be stressed at this point that the experiments reported in Figure 1 were performed using water as the transporting liquid. Rutin is an ionic substance due to presence of hydroxyl groups in this molecule, and its partition coefficient between water and organic phase depends on the pH. Thus, it is necessary to examine the influence of pH on the MSPD process of rutin. Figure 2 presents the changes in the MSPD yield of rutin from the flowers versus buffer pH. The curves were obtained for three different buffers in the pH range corresponding with their maximum buffer capacity and using the same ratio of m_s:m_p = 4:1. As appears from the plots, the concentration of hydrogen ions significantly influences the MSPD process of rutin. Taking these dependences into account, it was decided to repeat the previous series of MSPD experiments (see Figure 1) using buffers of different pH instead of water. Because the mobile phase in HPLC contains acetic acid, mainly acetate buffers were used in the experiments.

As results from Figure 3, at higher pH (4.5 and 5.5) the shape of the $1/E_{(\%)} = f(m_p/m_s)$ dependences is the same as the shape of *curve A* in Figure 1 (when water was used in the MSPD process).

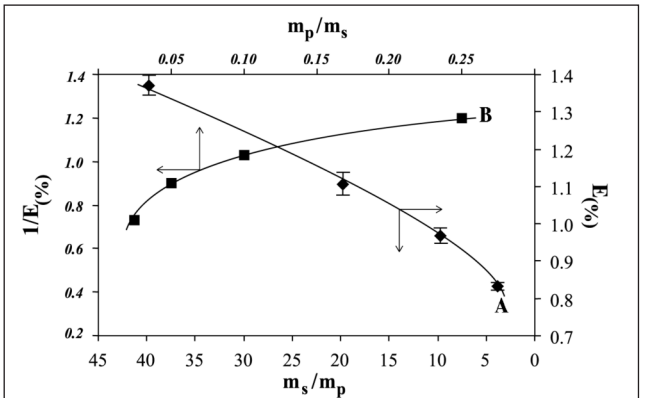

Figure 1. The dependence between the rutin yield (E(%)) and the mass ratio of sorbent to plant matrix (ms/mp) (curve A) and corresponding 1/E(%) vs. (mp/ms) function (curve B), both obtained for Sambucus nigra L. flowers using the MSPD technique. Water was a medium transporting the analyte from plant matrix to C_{18} sorbent. n = 5 for curve A. Curve B was plotted on the basis of the mean value taken from curve A.

However, at pH = 4.5 the dependence is more flat (see the proper scale). A higher concentration of hydrogen ions (pH = 3.6) results in the linearity of the discussed relationship in the applied range of mass ratio of sorbent to plant matrix. The linear run of the $1/E_{(\%)}$ *vs.* m_p/m_s plot suggests that MSPD, if carried out in proper conditions, can be described similarly to a simple non-disturbed liquid–liquid extraction. Consequently, the limit of this function (when $m_p/m_s \rightarrow 0$) should be equal to a reciprocal value of the total amount of rutin in the flowers of *Sambucus nigra* L. The linear equation describing the straight-line from Figure 3 is given in the first line of Table I. According to this equation, the total rutin amount ($E_{(\%)total}$) in the examined flowers equals 2.90%.

The comparison of this value with the established true value of rutin amount in *Sambucus nigra* L. is the simplest way to verify the applicability of MSPD for the estimation of the total rutin amount in plant matrices and accuracy of the measurement. The true total rutin amount in the investigated flowers of *Sambucus nigra* L. was determined by performing multiple PLE on the same sample under the optimised PLE conditions (80% MeOH, temperature 100ºC, static extraction time 10 min, pressure 100 bar) until no rutin was detected by HPLC. The results of these investigations are listed in Table II. The limit of detection of rutin represented less than 0.21% of the total amount of the compound found in the examined flowers. As can be calculated from the Table II, almost 99% of the estimated amount of rutin is extracted from the flower matrix in the first two cycles of the PLE processes. The comparison of both calculated values (2.90% obtained from linear MSPD dependence in Figure 3, or from the first equation in Table I and 2.88% obtained from multiple PLE – Table II) shows that they are very similar. Assuming that the value obtained by multiple PLE (treated as an exhaustive extraction) is a true one, it can be conclude that MSPD carried out in the conditions giving the linear plot $1/E_{(\%)} = f(m_p/m_s)$ is useful for analyte estimation in plant matrices, although none of the individual MSPD experiments have given the true value.

The rutin amount calculated from one cycle PLE carried out at various ratio of the flower mass to the extrahent volume (22–38) equals 2.95%. The last value is very close to the analyte amount revealed either by MSPD (Table I) or by multiple exhaustive PLE (Table II), which confirms independently the accuracy and reliability of the investigated MSPD way.

Looking for the reasons of non-linearity of $1/E_{(\%)} = f(m_p/m_s)$ function corresponding to MSPD, it was found that in this process the partition coefficient for rutin depends on pH. As results from Figure 3, the MSPD partition coefficient of rutin, for the range of mass ratio between 4–40, is constant at pH = 3.6. At a higher pH (4.5 and 5.5) the partition coefficient in this range is not constant. The shape of the corresponding $1/E_{(\%)} = f(m_p/m_s)$ dependences is not linear. In order to obtain a straight line for $1/E_{(\%)} = f(m_p/m_s)$ function at a higher pH, another greater (m_s/m_p) range should be examined (remembering that the obtained result will be less precise due to greater experimental error). The dotted line with white triangles in Figure 4 corresponds to the MSPD process of rutin, performed using acetate

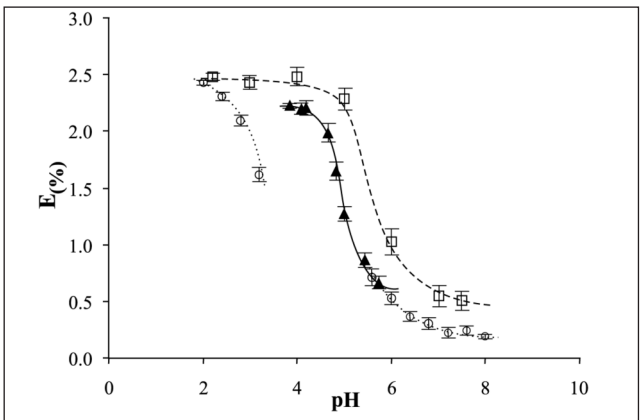

Figure 2. Rutin yields (E(%)) from Sambucus nigra L. flowers estimated using MSPD vs. pH for acetate buffer (solid line from black triangles), citrate buffer (dashed line with white squares), and phosphate buffer (dotted line with white circles). M_s/m_p = 4:1 was used in all the experiments.

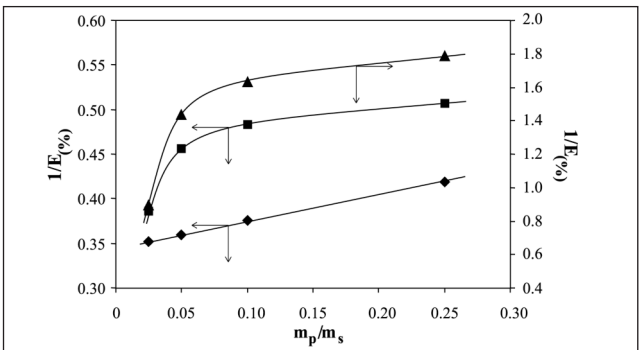

Figure 3. (1/E(%)) vs. (mp/ms) dependence obtained for rutin from Sambucus nigra L. flowers using MSPD and acetate buffer of pH = 3.6 (diamonds), pH = 4.5 (squares), and pH = 5.5 (triangles).

Table II. Rutin Yields (in % w/w) Obtained in Subsequent PLE Processes of the Same Sample (0.4 g) of Sambucus nigra L. Flowers

Number of PLE cycles	Rutin yield (% w/w)
1	2.5869
2	0.2638
3	0.0098
4	0.0077
5	0.0068
Σ	2.875 ≈ 2.88

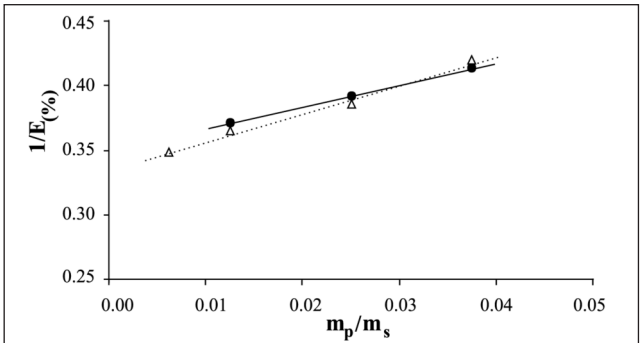

Figure 4. Reciprocal of rutin amount estimated in Sambucus nigra L. flowers versus the mass ratio of plant to sorbent (mp/ms), which were applied in MSPD (dotted line with white triangles corresponds to acetate buffer pH = 4.5; solid line with black circles corresponds to phosphate buffer pH = 2.0).

buffer of pH = 4.5 and a considerably greater mass ratio of sorbent to flower (m_s/m_p). The dependence can be described by the equation given in the second row of Table I. As shown in the Table, almost the same rutin amount (2.97%) in the flowers was found what confirms the last statement.

Figure 4 (a solid line with black rings) and Table I (third row) present additionally the data for the examined flowers obtained using MSPD with phosphate buffer at pH = 2.0. About 2.9% of rutin in *Sambucus nigra* L. flowers is confirmed again. It should be stressed that the calculated value is equivalent to the literature data concerning *Sambucus nigra* L. (39,40).

Conclusion

The main idea of this paper is to show that it is possible to find an estimate for the true concentration value of the analyte by means of MSPD based on the law of the partition process (e.g. liquid–liquid extraction) and on the properly adapted equation describing this process. It shows the need to carry out MSPD procedures in the conditions in which the 1/E versus sorbent to plant mass ratio is linear. The obtained results additionally show that PLE, which recently is more and more frequently used in the analysis of herb material and which requires the application of the expensive PLE equipment, can be replaced by the relatively cheap and effective MSPD process, which is less time-consuming than the multiday, exhaustive extraction in Soxhlet.

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