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**Extraction methods for the preparation of samples for
the analysis of complex matrices**

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Abstract

The dissertation thesis is formally divided in two parts. The first part is devoted to the theoretical foundation. When the characteristics of the studied matrices and the reasons for their analysis are first described. Next, attention is paid to the analytes present in these matrices, a description of the properties of the given group of analytes and methods of their common analysis. Subsequently, the classically used and modern methods of sample preparation, mainly liquid-phase and solid-phase extraction techniques, are presented.

The second part is devoted to the description of individual papers published during the doctoral program. Specifically, the identification of volatile organic compounds in several wood species and the use of their profile for statistical analysis to distinguish the origin of larch wood. Subsequently, the findings of a review on the importance and function of ionic liquids in composite materials are summarized. Last, the use of aqueous two-phase systems for extraction and concentration of contaminants in wastewater is described.

Abstrakt

Disertační práce je formálně rozdělena do dvou částí. První část je věnována teoretickým základům, kde jsou popsány charakteristiky studovaných matric a důvody pro jejich analýzu. Dále je pozornost věnována přítomným analytům v uvedených matricích, popisu vlastností dané skupiny analytů a způsobům jejich běžné analýzy. Následně jsou uvedeny klasicky používané a moderní způsoby přípravy vzorku, především extrakční techniky na bázi kapalně a tuhé fáze

Druhá část je věnována popisu jednotlivých prací publikovaných během doktorského studia. Konkrétně se jedná o identifikaci těkavých organických látek v několika druzích dřev a využití profilu těkavých organických látek pro statistickou analýzu s cílem rozlišení původu modřínového dřeva. Následně jsou shrnuty poznatky ze souhrnného článku týkajícího se významu a funkce iontových kapalin v kompozitních materiálech. Jako poslední je popsáno využití vodných dvoufázových systému pro extrakci a zakoncentrování kontaminantů v odpadních vodách.

Keywords

VOC, SPME, PCP, ABS, chromatography

Klíčová slova

VOC, SPME, PCP, ABS, chromatografie

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Goals of the doctoral dissertation

To identify individual compounds in the volatile profile of different wood samples obtained by solid phase microextraction and gas chromatography coupled with mass spectrometry.

To use the profiles of volatile organic compounds obtained by HS-SPME-GC as a "fingerprint" to distinguish the origin of larch wood without the need for prior identification of individual substances.

To provide information on the role and contribution of ionic liquids in composite materials; characterize the different groups of composite materials and summarize their representation.

To optimize a microextraction technique based on an aqueous two-phase system and apply it to the determination of pollutants (personal care products) in wastewater by liquid chromatography.

1. Introduction

Analytical chemistry has undergone a dramatic change since the middle of the last century when classical analytical methods were gradually replaced by instrumental methods. There has been a significant reduction in chemical consumption and improvements in sensitivity. Despite these advances, some problems remain constant, such as the need to achieve increasingly sensitive methods or the determination of selected analytes in complex As a result, sample preparation, which includes isolation of target analytes, concentration to reduce detection and quantification limits, and removal of interfering compounds, remains an essential part of analytical methods [1].

The development of sample preparation methods is influenced by the requirements of green analytical chemistry, which are the use of less toxic and biodegradable substances, reduction of chemical use, energy consumption, waste generation, and elimination of potential sources of exposure to humans working in the laboratory [2]. Furthermore, these techniques are required to be simple, inexpensive, and rapid to provide acceptable selectivity and recovery. This leads to the need for miniaturization and automation of known extraction techniques. An extraction technique called solid-phase microextraction meets most of these requirements and is only gaining popularity with time [3]. Another key step is the discovery and more detailed study of modern materials, which include, for example, ionic liquids. Ionic liquids are steadily gaining in popularity, promising low toxicity, high efficiency and the ability to vary selectivity [4].

2. Design of the Extraction Process for Characterization of Volatile Profile of Stem Wood by Solid-Phase Microextraction

In this publication, a method for the isolation of volatiles from wood samples using headspace solid phase microextraction (HS-SPME) was developed. The method was optimized using central composite planning. The optimization of extraction temperature and extraction time was performed in the range of 40-120 °C and 10-90 min, respectively. The optimized extraction method was combined with GC-MS to separate and identify volatile compounds in wood samples of seven tree species. These were deciduous trees (acacia, alder, beech, elm, larch, maple and oak) commonly found in Central Europe. A total of 185 organic compounds were identified in the volatile matter profiles, which were further classified according to compound type into 16 groups.

2.1. Statistical evaluation of the experimental design

Since the studied matrix has a large number of volatiles of different polarities, a commercially available SPME fiber with divinylbenzene/carboxen/polydimethylsiloxane sorbent (DVB/CAR/PDMS) was selected. Optimization of the extraction temperature and extraction time of the SPME method was performed in 12 experiments (8 plus 4 central points), which were determined by central composite planning. The individual experiments were performed in random order, while the central points were evenly distributed throughout the design to avoid systematic error.

Given the aim of this work, to extract and identify as many compounds as possible, the total number of peaks (NoP) in the resulting chromatograms was chosen as the variable of interest. The highest NoP was achieved under the following conditions: extraction temperature ranged from 95 °C to 115 °C and extraction time from 10 to 20 min. The optimum extraction conditions were selected as an extraction temperature of 105 °C and an extraction time of 15 minutes.

2.2. Analysis of real samples

The developed HS-SPME method was used to analyse the VOC profiles of seven wood species. All identified substances were categorised into 16 groups based on their structure, see Table 1. as can be seen, each group was present in at least two samples.

Table 1 Chemical composition (% rel.) of VOC profile. Adapted from [5].

	<i>Acacia</i>	<i>Alder</i>	<i>Beech</i>	<i>Elm</i>	<i>Larch</i>	<i>Maple</i>	<i>Oak</i>
<i>Alcohols</i>	1,20	n.i.	<0,1	<0,1	n.i.	0,11	n.i.
<i>Carbonyls</i>	27,84	19,71	1,68	2,73	0,42	8,32	4,35
<i>Acids</i>	6,28	2,12	5,12	0,19	0,98	0,35	29,64
<i>Esters</i>	12,73	13,54	1,95	8,34	n.i.	9,22	2,68
<i>Monoterpenes</i>	n.i.	n.i.	0,19	n.i.	3,74	<0,1	0,11
<i>Oxygenated monoterpenes</i>	1,64	1,36	1,44	n.i.	4,39	0,17	0,57
<i>Sesquiterpenes</i>	n.i.	n.i.	n.i.	6,28	26,23	n.i.	n.i.
<i>Oxygenated sesquiterpenes</i>	2,11	5,06	1,62	33,62	3,50	6,24	0,61
<i>Diterpenes</i>	0,57	0,62	4,07	0,19	13,08	n.i.	3,17
<i>Oxygenated diterpenes</i>	0,26	0,76	10,34	n.i.	18,13	1,00	1,96
<i>Aliphatic hydrocarbons</i>	21,53	12,85	43,03	5,97	0,48	53,07	24,62
<i>Aromatic hydrocarbons</i>	0,15	0,23	1,07	10,48	n.i.	1,64	0,64
<i>Phenolics</i>	0,34	0,42	2,88	<0,1	1,04	0,57	2,31
<i>Apocarotenoids</i>	0,59	0,91	n.i.	0,22	n.i.	0,63	n.i.
<i>Lactones</i>	n.i.	n.i.	n.i.	n.i.	n.i.	0,29	0,43
<i>Miscellaneous</i>	0,37	0,33	<0,1	<0,1	0,16	0,32	0,13

n.i. – not identified

3. Volatile compounds fingerprinting of larch tree samples for Siberian and European larch distinction

The optimized HS-SPME method (see Chapter 5.1) combined with gas chromatography with flame ionization detector was used to differentiate larch wood from Central Europe and Siberia based on different VOC profiles. The observed variability in VOC content and abundance is influenced by many factors, including genetic, environmental and local spatial influences. The proposed method was used to measure the VOC profiles of 82 samples of European and Siberian larch. Individual VOC profiles were characterized using retention indices (RI) and peak areas, which were then statistically analyzed using the orthogonal projection to latent structures (OPLS) method. This procedure was able to distinguish the correct origin of all 82 larch wood samples.

3.1. Selecting a suitable SPME fiber

Following the manufacturer's recommendations for the extraction of non-polar substances, two SPME fibers were selected to test the suitability of VOC extraction from larch wood samples. From the results considering NoP and total peak area (TPA) shown in Table 2, it is evident that the DVB/PDMS/CAR blended fiber provided higher NoP and higher TPA compared to the PDMS fiber which showed lower efficiency under all conditions. For this reason, DVB/PDMS/CAR fiber was selected for the purpose of this work.

Table 2 Comparison of the results of HS-SPME-GC-FID analyses obtained with PDMS and DVB/CAR/PDMS fibers, expressed in terms of NoP and TPA dependent on the conditions. Adapted from [74].

Extraction temperature [°C], Extraction time [min]	100 μ m PDMS		50/30 μ m DVB/PDMS/CAR	
	NoP	TPA	NoP	TPA
30, 50	65	3,61E+06	163	8,48E+06
30, 90	69	1,21E+06	183	9,30E+06
75, 90	167	1,43E+06	297	9,18E+06
120, 90	289	1,29E+06	362	7,57E+06

3.2. HS-SPME optimization parameters

The effect of extraction temperature and extraction time on NoP and TPA was tested. Statistica 12 statistical software (StatSoft CR, Prague, Czech Republic) was used to obtain response surface models for NoP and TPA along with critical values for extraction temperature and extraction time. The critical values for the TPA model were 72.3 °C and 94.5 min (see Fig. 1b). In the case of NoP (Fig. 1a), the maximum was not reached, which means that the critical values of the independent variables lie outside the defined range of the extraction parameters and therefore could not be detected.

The reason for this phenomenon can be found after approximately dividing the VOC profile into three regions: the first region with RI up to 1260, the second region with RI from 1261 to 1785, and the third region with RI higher than 1785. For each mentioned region, a dependence on extraction temperature and time was constructed (Fig. 2). By examining the results, it was found that the constant growth of NoP was mainly due to the substances with higher RI.

Using the values from the TPA model, fewer substances would be extracted in larger amounts and some substances with higher RI could be omitted completely. To avoid this, a suitability surface model (Fig. 1c) was constructed by combining the NoP and TPA models, which already reaches a maximum. Based on this model, the optimal extraction parameters were selected: extraction temperature 115 °C and extraction time 110 min.

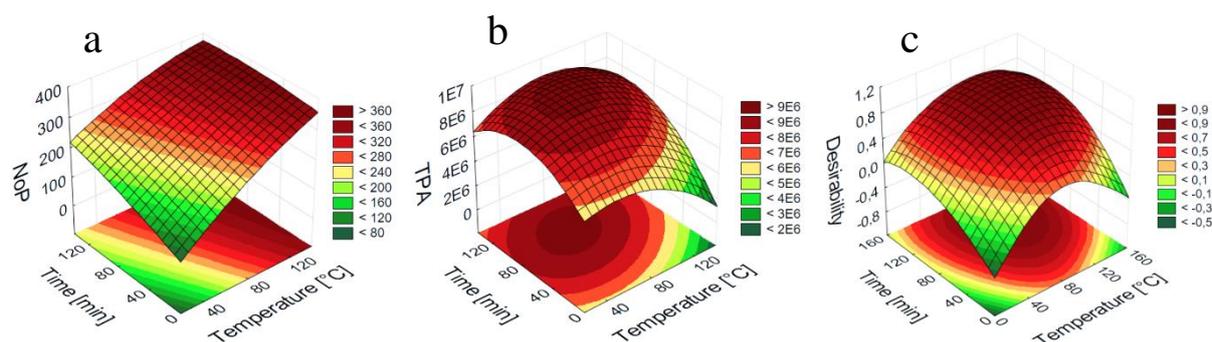


Figure 1 Graphs showing the effect of extraction temperature and extraction time on NoP (a) and TPA (b). The plot of the suitability area (c) shows a combination of the two previous plots. Adapted from [74].

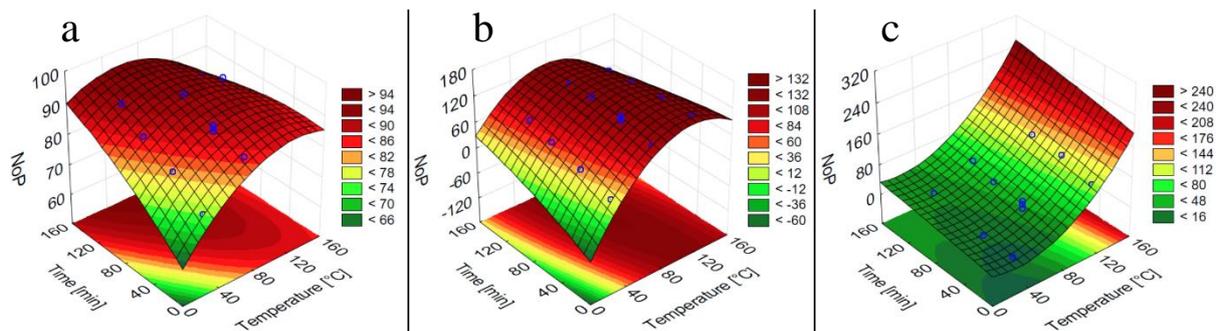


Figure 2 NoP plots for the first region with RI up to 1260 (a), the second region with RI from 1261 to 1785 (b), and the third region with RI higher than 1785 (c). Adapted and translated from [74].

3.3. Statistical evaluation of aroma profiles using OPLS

All 82 larch wood samples were analyzed by the optimized method HS-SPME-GC-FID. The prediction of each sample was performed as follows. After integration of all chromatograms, only peaks with an area ten times larger than the noise value were selected for the dataset. The data were transformed to obtain a symmetric distribution. The homogeneity of the predictors was tested using Hotelling's T2 statistic and the significance of the predictors was tested using the Variability Importance statistic. Then the following parameters were calculated:

- component loadings for each variable to determine the correlations of the relevant predictors with the OPLS predictive component and the % of variability that can be explained by the model,
- regression coefficients for the multiple regression model to determine the relationships between larch origin (variable Y) and the predictors (variable X),
- predicted values for all observations,
- probability of Siberian and European origin for all individual subjects,
- sensitivity and specificity of prediction.

The OPLS model had one predictive component and two orthogonal components. Figure 3 shows a "score scatter plot" informing about the between-group (predictive component, X-axis) and within-group (first of the two orthogonal components, Y-axis) variation. It shows the low inter-observer variation in the European larch sample group compared to the Siberian group, i.e. the greater differences in the VOC profile of the Siberian samples than European. The "loading scatter plot" (Fig. 4) shows a greater number of predictors correlating with the dependent variable on the left-hand side, indicating that a greater number

of substances act as predictors for Siberian larch (389 out of 576) compared to European larch (187 out of 576).

The distribution of the coefficient vectors of each predictor is worth highlighting (Figure 5). These coefficients express the strength of the correlation of the dependent variable with the predictors. Notable is the region with RI up to 1300, i.e., the region of more volatile substances, where a large majority of substances are characteristic of Siberian larch, which makes this region very important for their classification. The absence of these lower boiling point substances in the aromatic profile of European larch may be due to different living conditions, such as higher temperature, more nutrients or more sunshine.

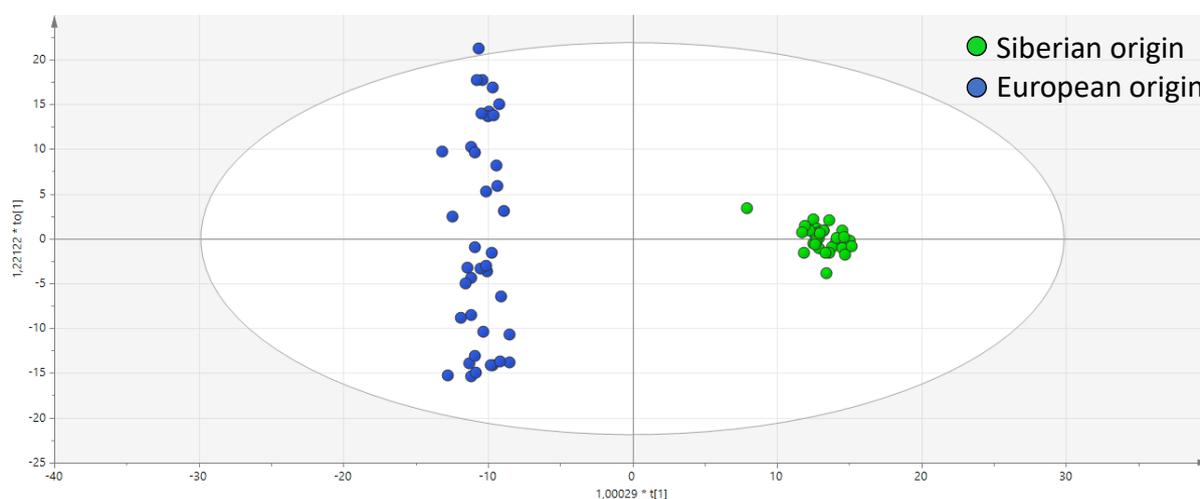


Figure 3 Scores scatter plot summarizing the relationships between observations. X-axis, relationships between groups of Siberian (left group) and European (right group) larch samples are represented by the predictive component. Y-axis, relationships within groups are represented by the first orthogonal component. Adapted from [6].

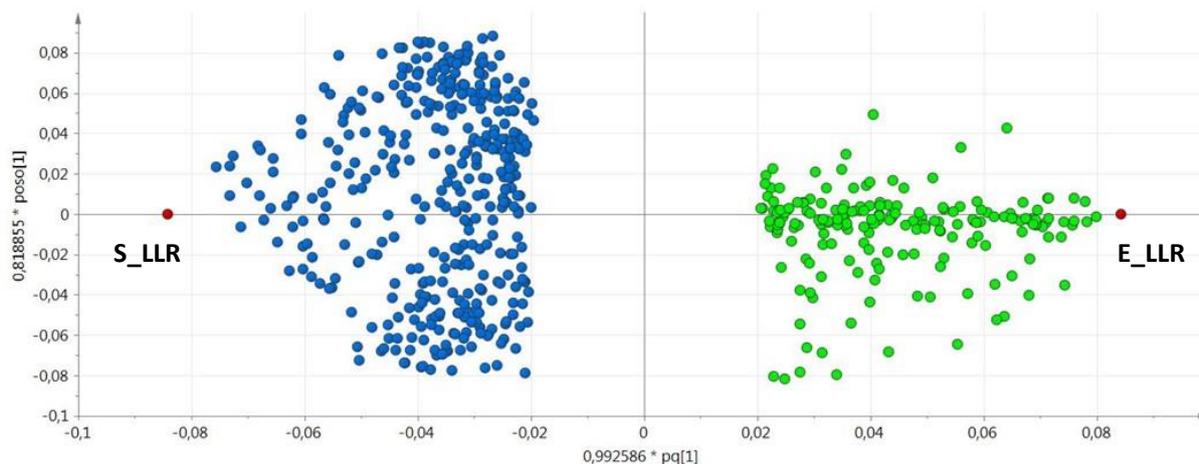


Figure 4 Loading scatter plot shows the relationships between the X- and Y-variable predictive components (X-axis) and the first orthogonal component (Y-axis). Adapted from [6].

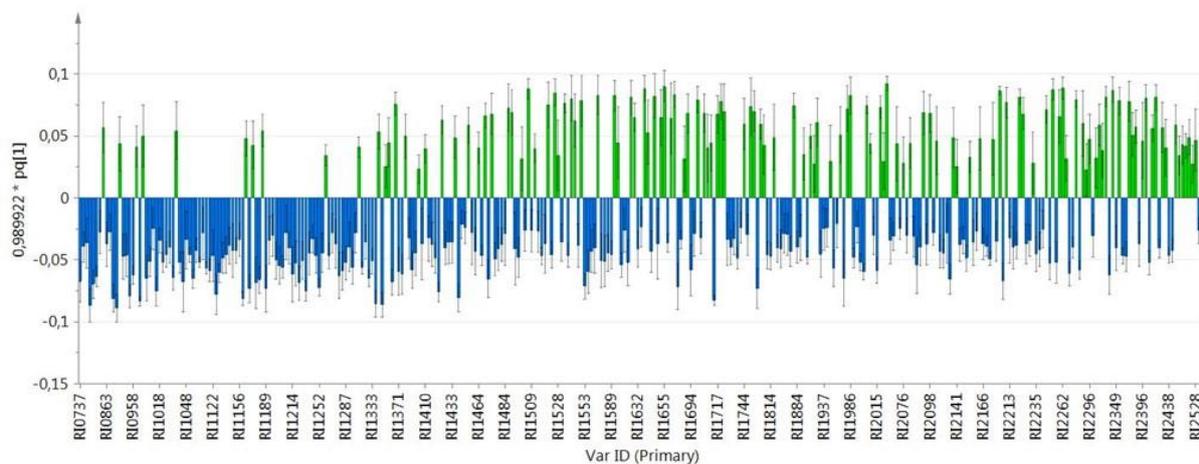


Figure 5 Coefficient plot shows the correlation of the probability of European origin of the larch sample to the individual peaks characterized by the retention indices. Predictors with non-significant (according to error bars, 95% confidence interval) coefficients were excluded from the plot. Adapted from [6].

Figure 6 shows the predictive ability of the model using two plots comparing the actual values with the probability values that a subject from the working dataset is a Siberian/European larch. The final OPLS model demonstrated good discriminatory ability, with a sensitivity of 1.000 (0.910; 1.000) and specificity of 1.000 (0.890; 1.000) for predicting Siberian larch in the set of samples tested (inferred at the 95% confidence level). That is, that all positive subjects (i.e. Siberian larch) were flagged as positive and none of the negative subjects were flagged as false positives. The model's ability to predict itself was verified using a prediction dataset composed of sample data that were not used in the working dataset. That is, data that were not used for model calibration. Figure 7. shows that all 12 samples from the prediction dataset were

classified correctly. This demonstrated that volatiles extracted from larch wood using HS-SPME-GC-MS can be used as predictors to determine the origin of larch wood.

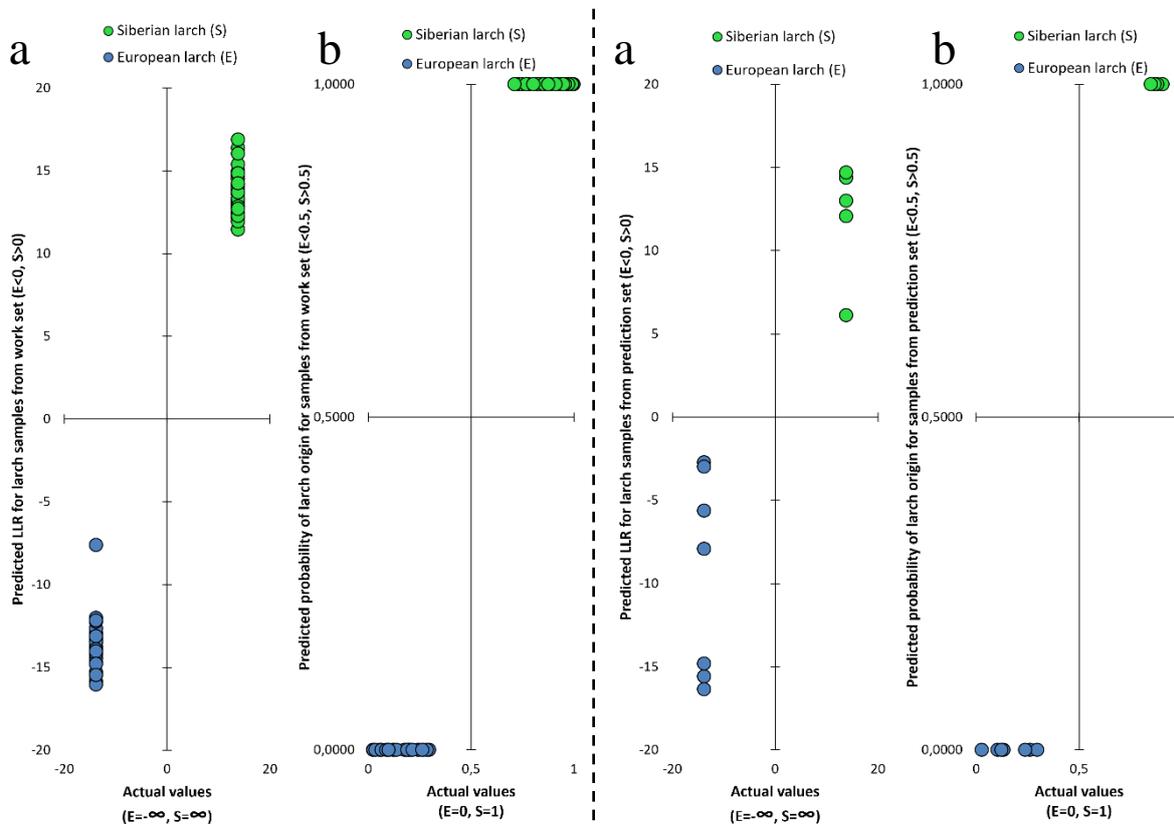


Figure 6 Resolution of Siberian/European larch (S/E) wood samples by OPLS statistical analysis of VOC profile obtained by HS-SPME-GC-FID. Samples from the working (calibration) dataset (31 European larch samples, 39 Siberian larch samples). Adapted and translated from [6].

Figure 7 Resolution of Siberian/European larch (S/E) wood samples by OPLS statistical analysis of VOC profile obtained by HS-SPME-GC-FID. Samples from the prediction dataset (7 European larch samples, 5 Siberian larch samples). Adapted and translated from [6].

4. Role of Ionic Liquids in Composites in Analytical Sample Preparation

Ionic liquids (ILs) are a group of unconventional salts with boiling points below 100 °C. Besides their negligible vapor tension at room temperature, high thermal stability and impressive dissolution properties, ILs are mainly characterized by their "tunability". Due to the almost infinite combinations of cations and anions, and the easy modifiability of their structure, ILs with very specific properties can be prepared. These properties have attracted a lot of attention, among others, as an extraction phase in sample preparation especially in liquid phase extraction methods. In contrast, due to the liquid nature of most ILs, their use as solid sorbents requires the preparation of derivatives as polymeric ILs or in combination with other materials as solid composite materials. Magnetic particles, carbon materials, polymers, silicon-based materials and metal-organic materials have been used in their preparation. This summary gives an overview of the preparation and application of IL-based composite materials for the preparation of analytical samples in the period 2017-2020 (March 2020), with a special focus on understanding the role and importance of ILs and other components in these composites.

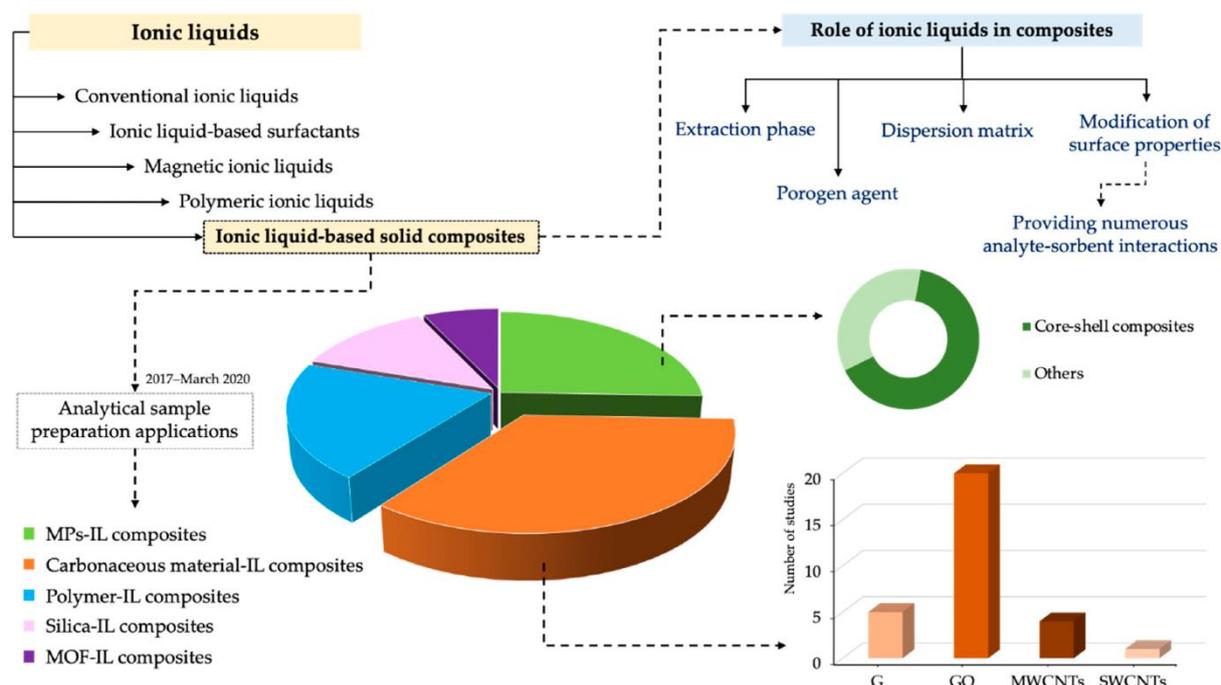


Figure 8 Overview of published studies in the period 2017-2020 (up to March). MPs - magnetic particles, MOF - metal-organic materials, G - graphene, GO - graphene oxide, MWCNTs - multiwalled carbon nanotubes, SWCNTs - single-walled carbon nanotubes. Adapted from [7].

As can be seen in Figure 8, ILs were most commonly combined with carbon materials, followed by polymer and magnetic composites. ILs were most commonly used in the role of modifying the surface properties of solid sorbents to improve extraction efficiency and selectivity by adding new interaction possibilities such as hydrogen bonding, π - π , electrostatic, hydrophilic, and hydrophobic interactions. The anchoring of ILs on the surface of the solid sorbents also improved the mass transfer and accelerated the extraction process due to the larger surface area of the resulting composite. In this sense, ILs are usually the main component of the composite responsible for the extraction properties.

In addition to surface modifications, ILs have been used in combination with other materials to prepare more complex and complicated structures, such as molecularly imprinted polymers with ILs as monomer, polymers with silicon-based materials, or "bucky gel" with carbon-based materials. In these cases, the IL was not only involved in the extraction of the analyte, but also played an important role in the final character of the composite material. Thus, the use of IL also improves the dispersion of solid materials to prepare more homogeneous sorbents and improves the porosity and permeability of the composite.

It is important to emphasize that the actual work should always consider the following: to compare the extraction efficiencies of the individual components as well as the final composite to clarify the necessity and usefulness of all the materials used in it. Virtually all publications in recent years have used conventional imidazole-based ILs with alkyl chains, mainly methyl or alkyl chains with 4-16 carbons. Although some authors have investigated the effect of IL structure on the nature and extraction efficiency of the resulting composite, their scope is limited to alkyl chains of different lengths and different anions. Thus, the "tunability" of ILs is of very limited use in this respect. In view of the large number of ILs that can be prepared and easily modified, it is important to move in this direction and it would be good to take advantage of this benefit to prepare selective sorbents with application-specific properties.

5. A green miniaturized aqueous biphasic system prepared with cholinium chloride and a phosphate salt to extract and preconcentrate personal care products in wastewater samples

A miniaturized ABS extraction and preconcentration method was developed consisting of compounds commonly used as food additives: choline chloride (ChCl) as the main extraction phase, K_2HPO_4 as the desalting element, water as the main component and also as the analytical sample. In order to achieve a high enrichment factor, miniaturization and adequate analytical performance, the point with the lowest amount of ChCl was selected in the phase diagram, corresponding to 1.55 % (w/w) ChCl, 59.5 % (w/w) K_2HPO_4 and 38.95 % (w/w) water. The method discussed was used in combination with high performance liquid chromatography with diode array detection (HPLC-DAD) for the determination of 9 PCP in wastewater samples. ABS-HPLC-DAD method (see Figure 9) showed high enrichment factors (up to 100) and quantitative extraction efficiencies for those substances that contain an OH group in their structure that is capable of forming hydrogen bonds with ChCl. For the paraben group and the UV-filter benzophenone-3, limits of quantification of up to $0,8 \mu\text{g}\cdot\text{l}^{-1}$ and extraction efficiencies between 66,4 % and 108 % were achieved (for concentration levels of 1,3 and $13 \mu\text{g}\cdot\text{l}^{-1}$). The method is characterized by the use of non-hazardous reagents and the absence of organic solvents throughout the sample preparation procedure, and is simple, inexpensive, HPLC compatible and highly efficient.

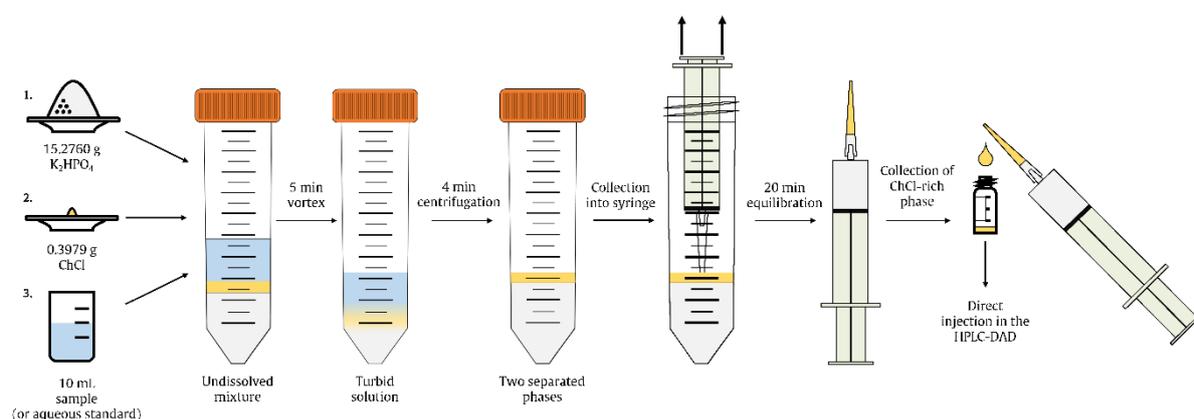


Figure 9 Schematic of the optimized ABS-HPLC-DAD method. Adapted from [8].

5.1. Coupling the ABS with HPLC-DAD

ABS composed of ChCl , K_2HPO_4 and water has already been described in the literature by various authors who report identical phase diagrams [9, 10]. For the actual use of the optimized system, a mixture of different PCP compounds in wastewater samples was selected to cover analytes with different structures and properties. The selected mixture of analytes included parabens, UV-filters, and disinfectants. Their determination was performed by HPLC-DAD. To check that the extraction phase did not interfere with the chromatographic separation and prevent correct identification, it was directly (without dilution) dosed into the HPLC system. The observation of a dominant signal at the beginning of the chromatogram indicates that ChCl , as the main component of the extraction phase, is not retained on the column. Figure 10 shows the chromatograms obtained after injection of the PCP standard in acetonitrile, the extraction phase after extraction using 10 ml of pure water and the extraction phase after extraction of 10 ml of aqueous PCP standard. After optimization of the HPLC-DAD separation, external calibration was performed. The observed analytical parameters of the instrumental method were used to determine the enrichment factor using ABS-HPLC-DAD.

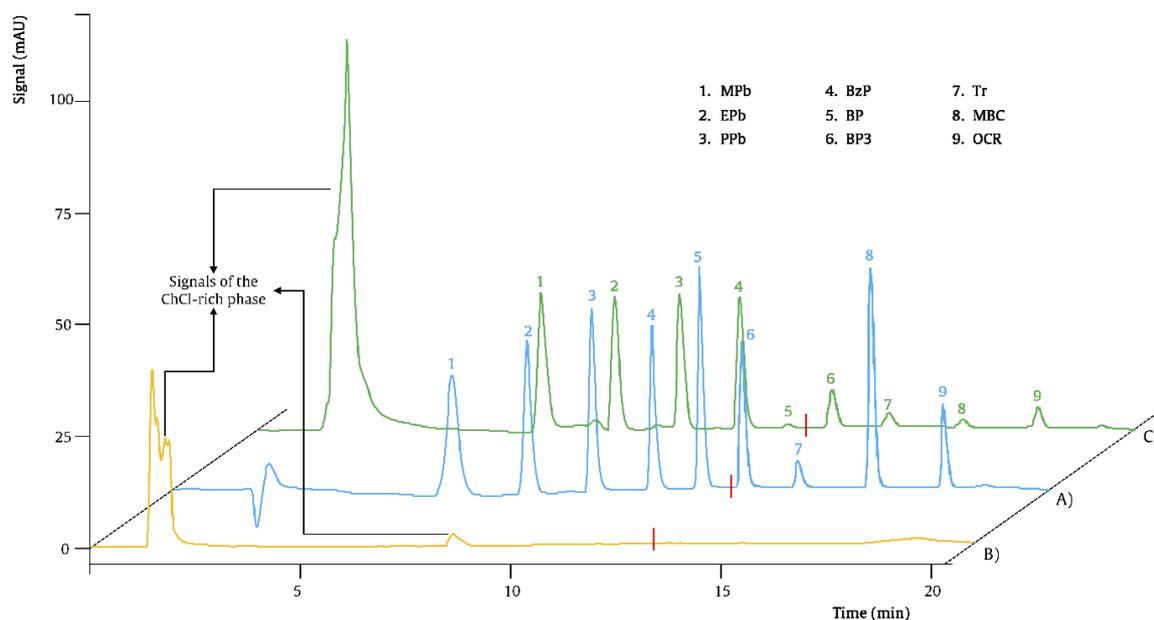


Figure 10 Representative chromatograms. (A - blue line) PCP standard in acetonitrile containing each analyte at a concentration of $3 \text{ mg}\cdot\text{l}^{-1}$, (B - yellow line) extraction phase after ABS extraction with 10 ml of ultrapure water, (C - green line) extraction phase after ABS extraction with 10 ml of aqueous standard containing all PCP analytes at a concentration of $34 \text{ }\mu\text{g}\cdot\text{l}^{-1}$. The red lines indicate the wavelength transition: 254 nm for analytes 1-5 and 289 nm for the remaining analytes. Adapted from [8].

5.2. Optimization of the ABS-based microextraction

The development of the phase diagram itself and the choice of a point in the biphasic region leads directly to the amount of reagents needed to achieve the desired preconcentration levels, which in itself represents an optimization of the ABS method. However, there are other experimental conditions that need to be considered as they can affect the extraction efficiency, such as the mixing time and equilibration time in ABS. Therefore, these parameters were optimized.

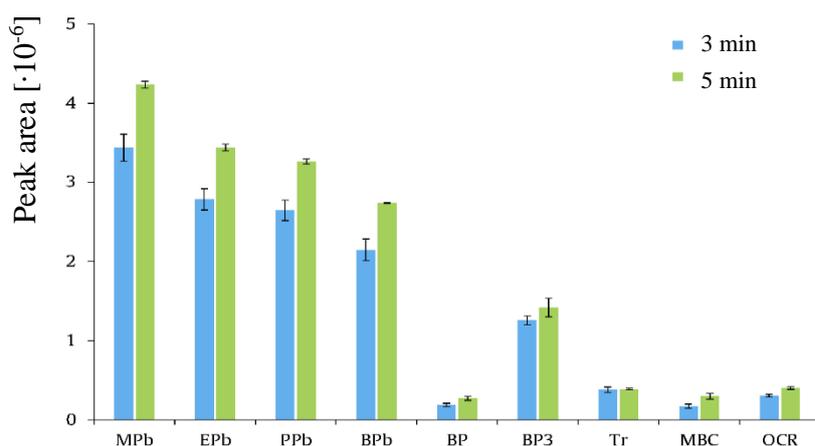


Figure 11 Graph showing the effect of mixing time on the extraction efficiency of the method ABS-HPLC-DAD. The vortex time of 5 minutes was chosen as the optimum mixing time. Adapted from [8].

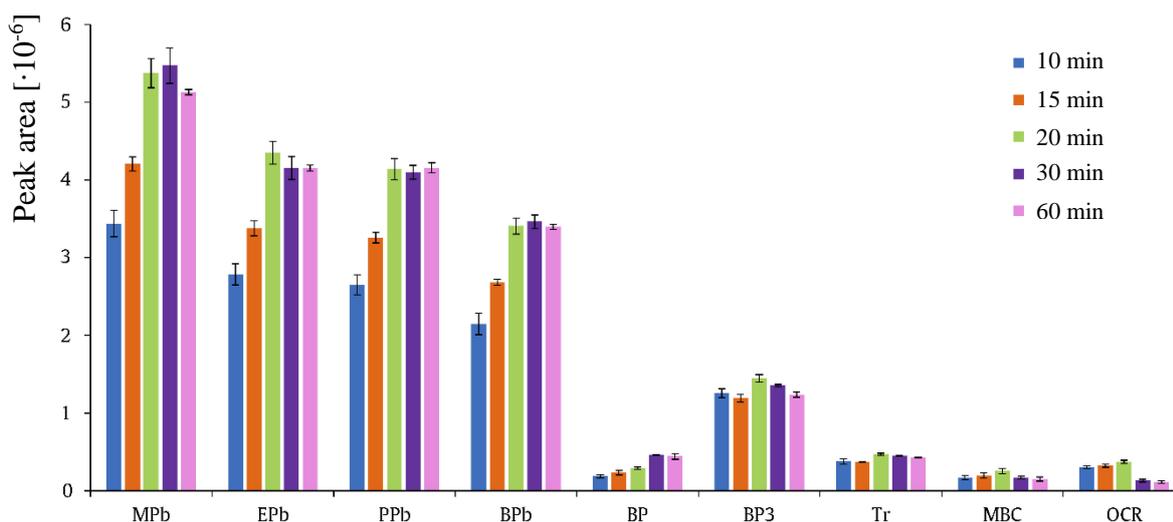


Figure 12 Graph showing the equilibration times for the extraction efficiency of the ABS-HPLC-DAD method. 20 minutes was determined to be the optimal time for ABS equilibration. Adapted from [8].

5.3. Analysis of wastewater samples

Wastewater samples from two different WWTPs were analyzed using the standard addition method. Calibration curves were prepared in the concentration range 0-22 $\mu\text{g}\cdot\text{l}^{-1}$. The obtained calibration curve guidelines were compared with those obtained using water standards to determine the matrix effect. It can be seen that the sensitivity was lower for the paraben group, while it increased slightly for the other substances. A matrix effect was found for all analytes in sample 1 and in sample 2 it was found for all but octocrylene.

Matrix effects in the case of UV-Vis detection may be due to extraction of other compounds present in the sample that have a higher affinity for the extraction phase, leading to suppression of the matrix effect, or coextraction and coelution of the interfering compound from the sample, leading to an enhancing matrix effect. Hence the recommendation for the use of internal standard, standard addition or other calibration methods that account for matrix effects when using the proposed ABS-HPLC-DAD technique to compensate for matrix effects in the case of complex samples.

In the case of determination of analytes in the sample, P-values were calculated to determine whether the section of the calibration curves obtained by the standard addition method was equal to zero. According to the results of the given statistical test, the intercept for many PCPs was different from zero, thus the concentration of these analytes could be calculated by extrapolation of the calibration curves. In general, it can be said that most analytes could be quantified in wastewater sample 2, with concentrations ranging from $0.4 \pm 0.1 \mu\text{g}\cdot\text{l}^{-1}$ for methylparaben and triclosan to $12 \pm 1 \mu\text{g}\cdot\text{l}^{-1}$ for benzophenone. In the case of wastewater sample 1, only some analytes could be quantified, ranging from $0.5 \pm 0.1 \mu\text{g}\cdot\text{l}^{-1}$ for triclosan to $9 \pm 1 \mu\text{g}\cdot\text{l}^{-1}$ for benzophenone.

It is worth noting that although benzophenone was the analyte with the highest concentration in both samples, it is also the analyte for which an amplifying matrix effect was observed. This suggests a possible coelution of other interfering compounds, which was confirmed by comparing the UV-Vis spectra of the sample and standard solution at retention times characteristic of benzophenone. The results for this substance should therefore be verified by other techniques (e.g. mass spectrometry).

6. Conclusion

This dissertation presents theoretical information about the studied matrices and the determined analytes. A more voluminous part is then devoted to sample preparation methods, liquid phase extraction, solid phase extraction and modern extraction materials.

The practical part summarizes the results obtained in the published articles. The first part deals with the volatile compound profiles and their identification in the wood of seven tree species and the use of VOC profiles as a "fingerprint" to distinguish the origin of larch trees. Next, the role of ionic liquids in composite materials has been studied and described, with a comprehensive classification and characterization of the different groups of composite materials in published papers from 2017 to March 2020. The last part focuses on an optimized aqueous two-phase microextraction method applied to the determination of daily use product substances in wastewater.

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8. List of Published Works

Articles

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Presentations

Jakub ŠULC, Testování desorpčních parametrů při použití HS-SPME k rozlišení evropského a sibiřského modřínu, Monitorování cizorodých látek v životním prostředí XX., Ovčárna pod Pradědem, 25. – 27. 4. 2018, 978-80-7560-154-4

Jakub ŠULC, Rozlišení sibiřského a evropského modřínu s využitím aromaprofilu jako otisku prstu, Monitorování cizorodých látek v životním prostředí XXI., Kojetín, 10. – 12. 4. 2019, ISBN 978-80-7560-234-3

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