Vortex-assisted ionic liquid based liquid-liquid microextraction of selected pesticides from a manufacturing wastewater sample

Research Article

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Abstract: The ionic liquid based vortex-assisted liquid-liquid microextraction (IL-VALLME) procedure was developed and validated for determination of four pesticides in a manufacturing wastewater sample: acetamiprid, imidacloprid, linuron and tebufenozide. The following ILs were tested as extractants: 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, 1-hexyl-3-methylimidazolium hexafluorophosphate, and 1-methyl-3-octylimidazolium hexafluorophosphate. The extraction efficiency and the enrichment factor dependencies on the type and amount of ionic liquids, extraction and centrifugation time, volume, pH and the ionic strength of the sample, were investigated. The concentration of pesticides in the aqueous and IL phases was determined by HPLC-DAD. The optimal conditions for extraction of the pesticides were determined: the aqueous sample volume of 10 mL with the addition of 0.58 g NaCl, 40 μL of the 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide as extractant, 2 min extraction under vigorous mixing applying the vortex agitator, and separation of the phases by centrifugation for 2 min at 1000 rpm. The calibration curves of the pesticides showed good linear relationship ($r^2 \geq 0.9996$) in the concentration range from 0.005 to 1.00 mg L^{-1}. Determined LOD values are 1.8, 2.3, 4.8 and 8.6 μg L^{-1} for Tebf, Linr, Acet and Imid, respectively. The optimized IL-VALLME was applied for determination of the pesticides in the pesticide manufacturing wastewater.

Keywords: Liquid-liquid microextraction • Pesticides • Room temperature ionic liquids • Wastewater
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1. Introduction

Sample preparation is an essential step in whole process of sample analysis. It includes removal of interfering compounds from complex matrixes and preconcentration of the targeted analytes. Various types of liquid-liquid and solid-phase extractions have been applied as the sample preparation step [1]. Recently, excellent review works of the sample preparation techniques were published emphasizing the importance of the sample preparation step in the analytical procedure [2-6]. Several liquid-phase microextraction techniques such as single drop microextraction [7], hollow fibre liquid-phase microextraction [8], dispersive liquid-liquid microextraction (DLLME) [9] and vortex-assisted liquid-liquid microextraction (VA-LLME) [10] have been introduced as alternatives to the traditional sample preparation techniques based on liquid-liquid extraction.

Most of sample preparation procedures based on the liquid-phase extraction consume organic solvents as extractants which are usually volatile or semivolatile at room temperature, flammable and non-environmentally friendly solutions. The aim of developing new sample preparation techniques is to overcome the drawbacks of the traditional extraction techniques, such as, long operation time, difficulty to automate, the use of large amounts of organic solvent etc. The main improvements of the miniaturized sample preparation techniques are exceedingly low solvent consumption (2-50 μL), flexible volume of sample (from a few mL to one liter), simple to operate and high enrichment factor, which leads to the low detection limit. The main disadvantage is the limited number of appropriate extractants.

The room-temperature ionic liquids (IL) represent a promising alternative for replacement of ordinary organic solvents in liquid-liquid extraction due to their distinctive

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physicochemical properties: negligible flammability and vapor pressure, broad liquid ranges, high solvation ability, high chemical and thermal stability, good extractability for various compounds and selectivity for organic and inorganic compounds [11-13]. Most ILs have organic cations, such as, imidazole, pyridine, pyrrolidine, phosphonium and ammonium. Anions can be inorganic, *e.g.* Cl⁻, Br⁻, I⁻, PF_4^-, PF_6^-, or organic, *e.g.* trifluoromethylsulfonate and bis[(trifluoromethyl)sulfonyl]imide. One noticeable characteristic of ILs is that changing of the cation and the anion can significantly influence their physicochemical properties. Because the properties of ILs can be tunable, they have been successfully applied in separation [14] and analytical chemistry [15,16].

Zhu *et al.* [17] applied for the first time IL based DLLME (IL-DLLME), which consists of a binary component solvent system (IL and aqueous sample), for determination of aromatic amines in water samples. After that, the application of IL-DLLME has been usually achieved using a ternary solvent component system (aqueous sample, IL and a polar disperser) [16,18]. A ternary solvent component system is associated with the use of relatively high volume of polar disperser solvents, which may lead to decrease of extraction efficiency due to increased solubility of the analytes in the aqueous solution. In order to replace the polar disperser in IL-DLLME and to reduce the consumption of volatile and toxic organic solvents, Zhao *et al.* [19] introduced a novel method ionic/ionic DLLME, with the water miscible IL as disperser. Further improvement of IL-DLLME was achieved by ultrasonic assisted IL-DLLME [20]. Use of IL as extractant in DLLME allows direct injection into HPLC, and can be well combined with the other analytical techniques: spectrophotometric, ICP and capillary electrophoresis [16]. The majority of IL-DLLME applications deal with water samples, but this technique has been successfully applied for biological and food samples [11,21].

The pesticide residues can reach the aquatic environment through the direct run-off, leaching, careless disposal of empty containers, equipment washing *etc*. Depending on their toxicity, the acceptable values in natural and drinking waters are in trace and ultratrace levels [3]. The concentration of pesticides in the wastewaters from pesticide manufacturing before releasing to the receiving water streams should be monitored. The maximum acceptable concentration of the pesticides in manufacturing wastewater before discharged into sewage or aquatic environment is 0.05 mg L⁻¹ [http://www.vilanova.cat/content/tramits/RE-AJU-49.pdf] [22-23].

The aim of this paper is to investigate the binary IL-VALLME technique and to combine it with HPLC for determination of the selected pesticides in the pesticide manufacturing wastewater sample. The effect of experimental parameters on the enrichment factor and extraction efficiency: type and volume of water immiscible IL, volume and pH of the aqueous solution, salt addition, extraction and centrifuge time, were investigated and optimized. After the optimization of the extraction parameters, the method was validated and applied for the determination of the selected pesticides at ppb level in the pesticide manufacturing wastewater sample.

2. Experimental procedure

2.1. Chemical and materials

The pesticides studied in this work are: acetamiprid (*N*-[(6-chloro-3-pyridyl)methyl]-*N'*-cyano-*N*-methyl-acetamidine), imidacloprid (*N*-[1-[(6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylureum) and tebufenozide (*N-tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide). All pesticides (95% purity) are obtained from Fitofarmacija a.d. (Zemun, Serbia). The commercial formulation of the pesticides: Tonus (acetamiprid 200 g kg⁻¹), Pozitron (imidacloprid 600 g L⁻¹), Galolin mono (linuron 500 g L⁻¹) and Rebus (tebufenozide 240 g L⁻¹) are also supplied from from Fitofarmacija a.d.. Methanol (HPLC grade), di-*n*-hexyl ether (DHE), tri-*n*-octylphosphine oxide (TOPO), dodecane, NaCl and ILs: 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_6MIM][($CF_3SO_2)_2$N]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([C_6MIM][PF_6]), and 1-methyl-3-octylimidazolium hexafluorophosphate ([C_8MIM][PF_6]) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

A stock solution of pesticide (200 mg L⁻¹ of each compound) was prepared in methanol, and was stable for at least 3 months when stored at -18°C. The working solutions were prepared by appropriately diluting the stock solution with Milli-Q deionized water (Millipore Co., Bedford, MA, USA).

2.2. Instrumentation

Chromatographic analysis was performed using Agilent 1100 liquid chromatograph (Agilent Technologies, Waldbroon, Germany) with Zorbax XDB-C18 column (4.6 × 250 mm, 3.5 µm particle size, Agilent Technologies, USA) and DAD detector at 254 nm. The flow rate was 0.7 mL min⁻¹ and an aliquot of 20 µL of the sample was injected into HPLC system.

The mobile phase consisted of methanol (A) and deionized water (B), using the following gradient profile: 0.0 min 43% A and 57% B, then 7 min 70% A and 30% B, and 20 min returned to the initial composition. The system is controlled by the Chemstation software.

2.3. Extraction procedure

In order to equilibrate the water content in IL, IL was stored in contact with ultrapure water for 1h before the experiment. The spiked water sample was put into a centrifugal tube. A predetermined volume of IL was quickly injected into a sample solution and vigorously shaken using a vortex agitator (Reax Top, Heidolph, Germany) at 2500 rpm (maximum setting). The fine droplets of IL were formed during the vortex shaking. For investigation of longer extraction time, the samples were the first vortex agitated 2 min, then shaking was continued using a laboratory shaker (Promax 2020, Heidolph, Germany) at 200 rpm. The IL was separated from aqueous solution by centrifugation (laboratory centrifuge MLW T 32, Janetzki, Leipzig, Germany) at 1000 rpm (RCF 145 g). The upper aqueous phase was decanted, and the IL phase was analyzed by HPLC.

Liquid-liquid extraction of the pesticides with 5% TOPO in DHE was performed by addion of 0.5 mL of this extractant into 5 mL spiked water samples and shaken 2 min on the vortex agitator and then 1 h on the laboratory shaker.

2.4. Calculations

The extraction efficiency (E) and the enrichment factor (EF) are used to evaluate the performance of the extraction. E is the fraction of the initial amount of the analyte (n_{aq}^{in}) removed from the aqueous phase:

$$E = \frac{\left(n_{aq}^{in} - n_W\right)}{n_{aq}^{in}} \qquad (1)$$

where n_W is the number of moles of analyte kept in the aqueous phase after extraction. EF is defined as the ratio of the concentration of analyte in IL after the extraction, C_{IL}, and the analyte concentration in the sample, C_{aq}^{in}:

$$EF = \frac{C_{IL}}{C_{aq}^{in}} \qquad (2)$$

The dissociation constants, pK_a, and n-octanol-water partition coefficients, $log\ P_{o/w}$, were calculated using the computer software ACD/Labs PhysChem Suite v12 (Advanced Chemistry Development Inc., Toronto, Canada). IL-water partition coefficient, $log\ P_{IL}$, of the pesticides is defined as the ratio of the equilibrium concentration of the pesticides in IL and in the aqueous phase. $log\ P_{IL}$ of the pesticide was determined under optimal extraction conditions.

3. Results and discussion

3.1. Selection of pesticides

In order to investigate the application of IL-VALLME for determination of pesticides in water samples, four pesticides belonging to the various chemical classes, were selected: acetamiprid, imidacloprid, linuron and tebufenozide. The main features of the pesticides which influence the extraction: pK_a, and $log\ P_{o/w}$ are given in Table 1.These pesticides have different polarity, $log\ P_{o/w}$ are ranging from 0.46 to 4.38, which directly influences their extraction [24,25]. The low polar compounds ($log\ P_{o/w} > 2$) can be easily extracted using the low polar organic solutions, such as, n-octanol and di-n-hexylether, while the extraction of more polar compounds ($log\ P_{o/w} < 2$) can be enhanced by using the polar extractants, such as, tri-n-octylphosphine oxide and tri-n-butyl phosphate [25].

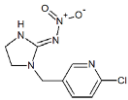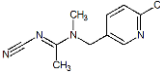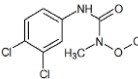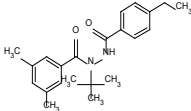## 3.2. Optimization of the extraction conditions

In order to extract simultaneously pesticides of different polarity and obtain high enrichment factor applying IL-VALLME, the parameters which affect the partition of the pesticides between two immiscible phases (aqueous and IL) were optimized.

3.2.1. Selection of IL

The selection of suitable IL among a large number of ILs that could be used for extraction of pesticides from the aqueous sample is based on their required properties: low solubility in water, good extraction ability for target analytes (*e.g.* imidazolium based ILs possess a high chemical affinity to compounds with one or more aromatics rings in their structure), higher density than water, and good chromatographic behavior [11,16,26]. For these reasons, three imidazolium based ILs were tested as the extractants: $[C_6MIM][(CF_3SO_2)_2N]$, $[C_6MIM][PF_6]$, and $[C_8MIM][PF_6]$. The main characteristics of these ILs are given in Table 2 [27,28]. They were chosen because of their relatively high hydrophobicity, suitable viscosity, low solubility in water and acceptable chromatographic behaviour. The maximum UV absorbance of the applied ILs is about 220 nm and their retention times under the applied chromatographic conditions are different than the retention time of the studied pesticides that allow their unhindered determination.

To evaluate the influence of the studied ILs on the extraction of the selected pesticides, a series of 5 mL aqueous samples spiked at 0.5 mg L^{-1} of each pesticide

Table 1. The main characteristics of the targeted pesticides.

Pesticide (shortcut)	Chemical class (activity)	Structure	pK_a	$logP_{o/w}$ pH 4-8	$logP_{IL/w}$		
					IL_1[c]	IL_2[d]	IL_3[e]
Imidacloprid (Imid)	Neonicotinoid (insecticide)		11.2[a]	0.46[b] 0.57[a]	1.58	1.32	1.80
Acetamiprid (Acet)	Neonicotinoid (insecticide)		0.7[a]	0.80[a]	1.60	1.46	1.28
Linuron (Linr)	Phenylurea (herbicide)		-	3.12[a]	3.25	3.21	2.86
Tebufenozide (Tebf)	Diacylhydrazine (insecticide)		-	4.38[b]	4.60	4.38	4.20

[a]determined by ACD/Labs PhysChem, [b][24], [c]IL_1: $[C_6MIM]$ $[(CF_3SO_2)_2N]$, [d]IL_2: $[C_6MIM]$ $[PF_6]$, [e]IL_3: $[C_8MIM]$ $[PF_6]$

Table 2. The main characteristics of applied ILs [27,28].

IL	Molecular mass	Density (g cm^{-3})	Viscosity 25°C (Pa s)	Solubility in water (g L^{-1})
$[C_6MIM][(CF_3SO_2)_2N]$	447	1.33	0.68	0.34
$[C_6MIM]$ $[PF_6]$	312	1.29-1.31	0.560-0.586	7.5
$[C_8MIM][PF_6]$	340	1.20-1.23	0.710	2

in triplicate were used for IL-VALLME. Fig. 1 shows the influence of applied ILs (≈ 45 µL) on the extraction efficiency of the investigated pesticides. To compare the obtained values, the extraction efficiency with 5% TOPO in DHE as extractant is also given in Fig. 1 [25].

It can be seen from Fig. 1 that E of the applied ILs depends on the constituents' both anions and cations. Higher E of more polar pesticides, Imid and Acet, was obtained with IL with $[(CF_3SO_2)_2N]$ anion in comparison with IL with $[PF_6]$ anion (cation was the same $[C_6MIM]$). This is because of higher polarity and lower solubility in water of $[C_6MIM]$ $[(CF_3SO_2)_2N]$ than $[C_6MIM][PF_6]$ [29]. The similar influence was not observed in the extraction of low polar pesticides. Comparison of E obtained with ILs with the different cations, $[C_6MIM]$ and $[C_8MIM]$ (anion was $[PF_6]$), shows less extraction efficiency in the cases of $[C_8MIM]$ cation for the three investigated pesticides (Acet, Linr and Tebf) probably due to higher viscosity of $[C_8MIM][PF_6]$. Only in the case of Imid was the highest E was obtained using $[C_8MIM][PF_6]$.

The obtained E for more polar pesticides (Imid and Acet) using the ILs are significantly higher than E using 5% TOPO in DHE. The extraction of low polar pesticides (Linr and Tebf) is almost complete using either pure IL or 5% TOPO in DHE. Only the $[C_8MIM][PF_6]$ is less efficient extractant giving extraction efficiency of 62 and 78% for Linr and Tebf, respectively, due to its higher viscosity.

The extraction mechanism of the selected pesticides with IL is the partitioning mechanism [11]. It is generally accepted that the transfer of the analyte from the aqueous phase to IL phase by the partitioning mechanism is similar to the one which occurs by using the traditional organic solvents. The $log P_{IL/w}$ of the studied pesticides was experimentally determined and given in Table 1. It was found that $log P_{IL/w}$ of more polar pesticides (Imid and Acet) is significantly higher than its $log P_{o/w}$, whereas, values of $log P_{IL/w}$ of low polar pesticides are similar to values of the corresponding $log P_{o/w}$. The highest $log P_{IL/w}$ of all the targeted pesticides was obtained using $[C_6MIM][(CF_3SO_2)_2N]$ as extractant.

The influence of methanol as a polar disperser solvent was investigated and also shown in Fig. 1 [16,19]. Methanol (0.5 mL) was added together with IL in the aqueous sample solution. All the other experimental conditions were the same as the ones already described in above paragraph. The methanol addition either had no influence on E of the selected pesticides (Linr) or slightly decreased E (Acet, Imid and Tebf). This
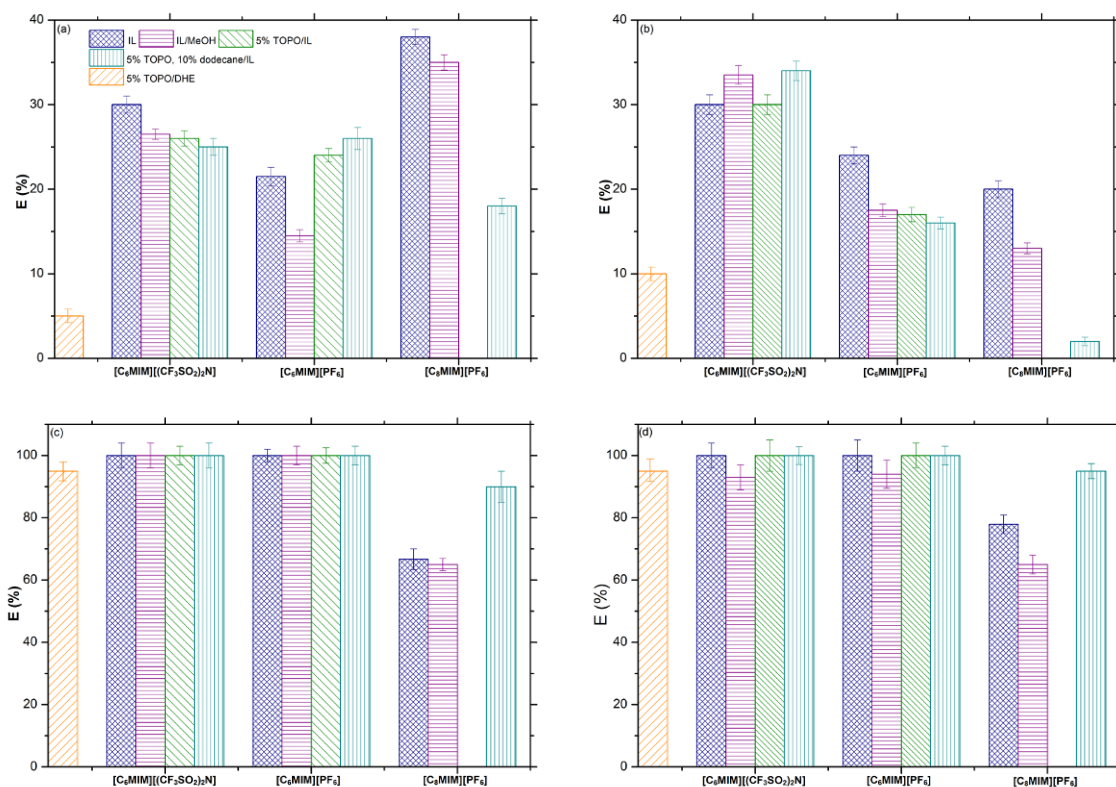
Figure 1. The influence of ionic liquids and different mixtures of extractant on extraction efficiency of (a) Imid, (b) Acet, (c) Linr and (d) Tebf. The error bars represent the standard deviation of experimental data.

is probably because of increased solubility of IL in methanol and increased solubility of the pesticides in the aqueous solution. Only in the case of the extraction of Acet with $[C_6MIM][(CF_3SO_2)_2N]$ extraction efficiency was increased with addition of methanol.

Further, in order to enhance extraction efficiency of the more polar pesticides TOPO was added in IL, due to the fact that TOPO as a polar extractant increases extraction of many organic molecules [25,30]. It is clear from Fig. 1. that addition of TOPO in $[C_6MIM][PF_6]$ slightly increases E of Imid and decreases E of Acet. Considering that TOPO (5%) is not dissolved in the $[C_8MIM][PF_6]$, dodecane (10%) was added to increase solubility of TOPO in this ionic liquid. The addition of dodecane and TOPO have increased the extraction of Linr and Tebf (Figs. 1c and 1d) in comparison with the use of pure $[C_8MIM][PF_6]$.

3.2.2. Effect of IL volume

Fig. 2 shows the effect of IL volume on the extraction efficiency and the enrichment factor for Imid (Fig. 2a) and Tebf (Fig. 2b). The experiments were performed by addition of various amounts of IL in aqueous sample (5 mL) spiked at 0.5 mg L^{-1} of each pesticide. It is clear from Fig. 2a, that by increasing volume ratio V_{IL}/V_{aq} from

0.0014 to 0.01, E of Imid is increasing and reaches the constant level for the interval of the V_{IL}/V_{aq} from 0.01 to 0.015. Acet shown the similar behavior. Only in the case of the extraction of Acet with $[C_6MIM][(CF_3SO_2)_2N]$, the maximum E was obtained using the largest investigated ratio of the V_{IL}/V_{aq} (0.015). The E of Tebf (Fig. 2b) was close to 100% for V_{IL}/V_{aq} ration higher than 0.004, except the lower maximal E was obtained for its extraction with $[C_8MIM][(PF_6)]$. The similar result was obtained for Linr. The highest EF was obtained using the minimal amount of $[C_6MIM][(CF_3SO_2)_2N]$ (7.3 μL). Taking into account optimum values of the extraction efficiency and enrichment factor, as well as, considering the experimental feasibility, the volume ratio V_{IL}/V_{aq} of about 0.008 and $[C_6MIM][(CF_3SO_2)_2N]$ as the extraction solvent was chosen in the subsequent experiments.

3.2.3. Effect of pH

The influence of sample pH on the extraction efficiency was investigated in pH range from 2 to 9 (adjusting with diluted HCl or NaOH) and covering laboratory (pH ≈ 5) and manufacturer wastewaters (pH ≈ 6.5 - 8.5). The pesticides were extracted by addition of $[C_6MIM][(CF_3SO_2)_2N]$ (40 μL) in aqueous sample (5 mL) at the spiked level of 0.5 mg L^{-1} of each pesticides. The
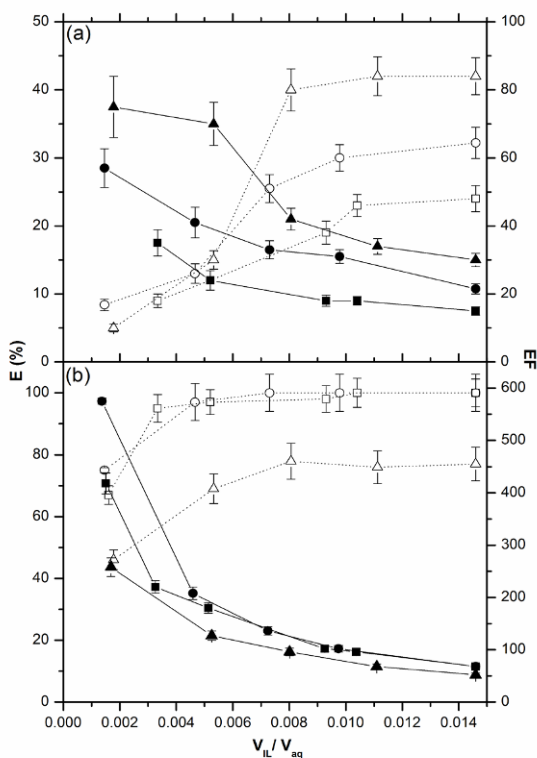
Figure 2. The influence of ILs volume on the extraction efficiency (left y-axis and open sign) and enrichment factor (right y-axis and full signs) of (a) Imid and (b) Tebf. Legend: □, ■ - $[C_6MIM][PF_6]$; ○, ● - $[C_6MIM][(CF_3SO_2)_2N]$; Δ, ▲ - $[C_8MIM][PF_6]$.

obtained E of the pesticides, expressed as the mean value of E obtained for all investigated pH ± standard deviation, were 28±2.8, 28.6±3.2, 90±3, and 100±2.5 for Imid, Acet, Linr and Tebf, respectively. These results indicate no influence of sample pH on the extraction efficiency of the targeted pesticides, except lower E (20%) was obtained for extraction of Acet at sample pH 2, probably due to hydrolysis of Acet.

3.2.4. Effect of the extraction and centrifugation time

Extraction time in liquid-liquid microextraction is defined as the time interval between addition of IL in the sample and the start of centrifugation. The time dependence of E on the studied pesticides was performed by addition of $[C_6MIM][(CF_3SO_2)_2N]$ (40 µL) in aqueous sample (5 mL) at the spiked level of 0.5 mg L^{-1} in the time interval from 2 to 120 min. Difference of E between the shortest and longest extraction time is from 0.7% for Linr up to 7.9% for Acet. Very short time of the extraction (2 min) was enough to reach the maximum extraction efficiency under the given conditions for all the investigated pesticides.

Centrifugation was applied for the separation of aqueous and IL phases; this step is often the most time-consuming in the extraction procedure. The effect of the centrifugation time on E of the pesticides was studied in the time range from 2 to 30 min at 1000 rpm. It was observed that the drops of the IL were fully condensed and separate from the aqueous phase in 2 min of centrifugation.

3.2.5. Effect of ionic strength

To investigate the influence of ionic strength on IL-VALLME of the pesticides, the aqueous sample solutions (5 mL, spiked at 0.5 mg L^{-1} of each pesticides) containing various concentration of NaCl (0 - 2 mol L^{-1}) were tested. Even though adding of NaCl increases the ionic strength of aqueous solution, the solvent strength of IL is maintained due to its good solvating properties [17]. In general, the addition of salt to aqueous phase decreases the solubility of analytes in the aqueous sample phase and leads to enhancement of their partitioning into the organic phase. Fig. 3 shows that EF of Acet increases from 35 to 58 with increasing NaCl concentration from 0 to 2 mol L^{-1}. The influence of NaCl on extraction of Imid, which exists as cation at applied pH (pH 5), is less pronounced. EF of less polar pesticides (Tebf and Linr), that were almost completely extracted from the aqueous solution, were not affected upon increasing of NaCl concentration up to 2 mol L^{-1}.

3.2.6. Effect of sample volume

Fig. 4 shows the influence of aqueous sample volume on the peak area of Linr and Tebf in IL after the extraction. The increasing of the V_{aq}/V_{IL} from 43 to 230, the peak area of the pesticides in IL increase due to increasing the total mass of analytes available for extraction. Further increasing of the ratio V_{aq}/V_{IL} the peak area decreased, probably because of the solubility of IL in water. Practically, the work with the volume ratio V_{aq}/V_{IL} being higher than 250 is difficult, because IL could not be separated from the aqueous phase.

The optimal experimental conditions found here were: the aqueous sample volume of 10 mL with addition of 0.58 g NaCl, 40µL of $[C_6MIM][(CF_3SO_2)_2N]$ as extractant, 2 min extraction under the vigorous mixing (2500 rpm), separation of the phases by centrifugation (2 min at 1000 rpm).

3.3. Analytical method performance

The linearity of the proposed method was determined by extracting under the optimal conditions, aqueous samples spiked at the concentration ranging from 0.002 to 1 mg L^{-1} for Linr and Tebf and 0.005 to 1 mg L^{-1} for

Table 3. The selected parameters of the calibration curves of the investigated pesticides and average relative recoveries (n=3) for tap and manufacturing wastewater sample spiked at 5 μg L^{-1} with Linr and Tebf and 9 μg L^{-1} with Acet and Imid.

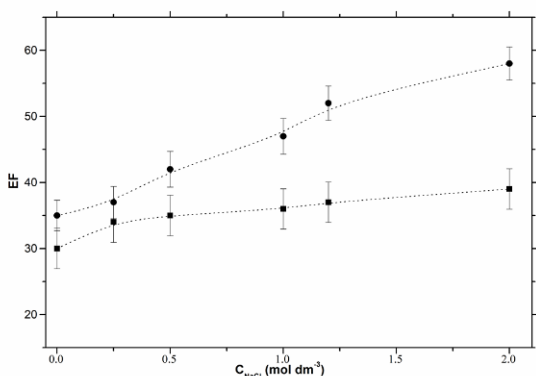| Pesticide	C (μg L^{-1})	r^2	EF	LOD (μg L^{-1})	Relative recoveries (%(RSD))	
					Tap water	Wastewater
Linr	4 - 500	0.9998	107	2.3	94 (4)	96 (11)
Tebf	4 - 500	0.9999	125	1.8	105 (3)	89 (9)
Acet	5 - 500	0.9997	55	4.8	102 (7)	91 (9)
Imid	7 - 500	0.9996	33	8.6	97 (5)	123 (7)

Figure 3. The influence of NaCl on the enrichment factor of Acet (•) and Imid (■). Extraction conditions: 5 mL aqueous solution of pesticides, concentration 0.5 mg L^{-1} of each pesticides, organic phase 40 μL of [C$_6$MIM][(CF$_3$SO$_2$)$_2$N], extraction time 2 min. The error bars represent the standard deviation of experimental data.
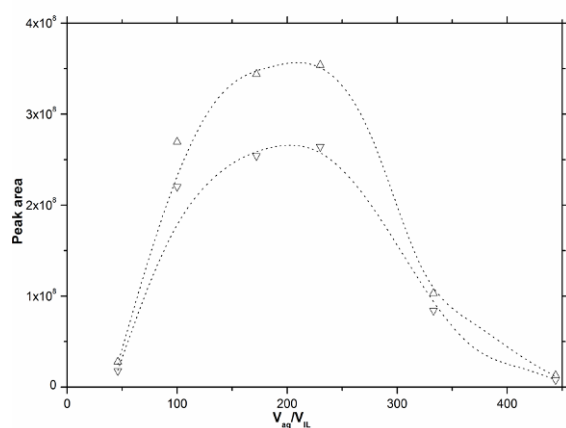

Figure 4. The influence of aqueous phase volume on the peak area of Linr (Δ) and Tebf (∇). Extraction conditions: concentration 0.5 mg L^{-1} of each pesticides, organic phase 40 μL of [C$_6$MIM][(CF$_3$SO$_2$)$_2$N], extraction time 2 min.

Acet and Imid. Each level was analyzed in triplicate. The studied pesticides exhibited good linearity with correlation coefficient (r^2) ranged from 09996 to 0.9999 (Table 3).

The selectivity of the proposed IL-VALLME was evaluated by the analysis of the blank samples (deionized and tap water). In the resulting chromatograms no peaks were detected at the retention times on of the studied pesticides. Also, the peak which derives from IL does not influence on the pesticides peaks for the applied wavelengths.

The reproducibility of the experimental procedure, expressed as the relative standard deviation (RSD), was evaluated for five replicate extractions at two concentration levels of 0.005 and 0.5 mg L^{-1}. The higher precision corresponding to RSD equal to 1.6, 3.7, 4.1, and 4.3%, for Tebf, Linr, Acet, and Imid, respectively, was obtained at the pesticides concentration of 0.5 mg L^{-1}. The lower precision corresponding to RSD equal to 3.6, 4.8, 8.5, and 10.6%, for Tebf, Linr, Acet, and Imid, respectively, was obtained at the pesticides concentration of 0.005 mg L^{-1}.

The limit of detection (LOD) was determined as the lowest pesticides concentration that produce chromatographic peak at a signal to noise ratio of three. The LODs range from 1.8 to 8.6 µg L^{-1}.

3.4. Real sample analysis

The proposed analytical method has been applied for determination of the pesticides in a pesticides manufacturing wastewater sample. The water obtained after equipment washing contains, besides active ingredients, various chemical compounds that increase pesticide activity, improves safety features and enhances handling qualities. The amount of built-in adjuvants in the pesticide formulations often surpasses 50% of the total weight of the product. These adjuvants can also interfere in chemical analysis of pesticides. Tap water spiked with commercial formulation of the targeted pesticides (5 µg L^{-1} of Linr and Tebf and 9 µg L^{-1} of Acet and Imid) represents the manufacturing wastewater. The relative recoveries, defined as the ratio of HPLC peak areas of the tap water spiked either with pure pesticides or with the pesticide formulation extracts to spiked ultrapure water extract, were calculated to evaluate
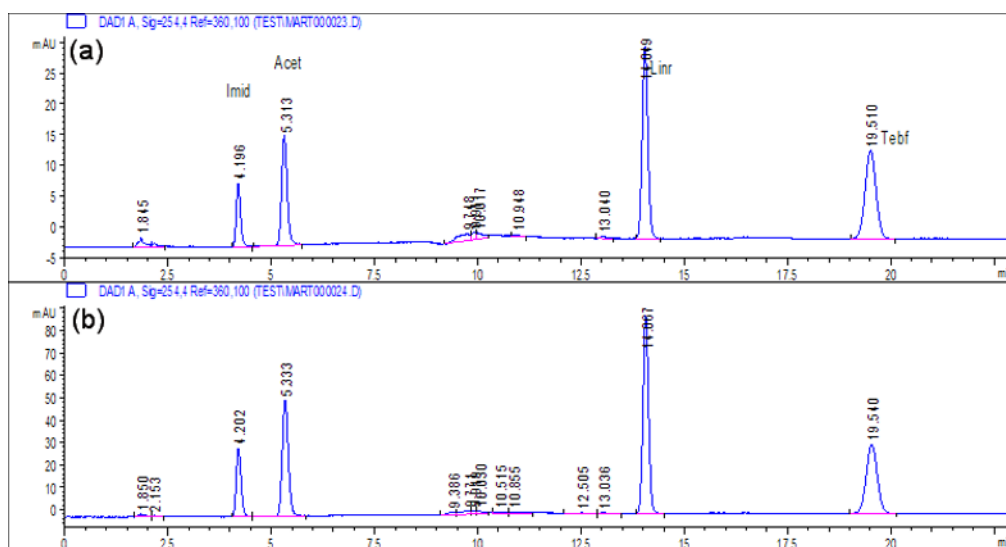
Figure 5. HPLC chromatograms obtained after IL-VALLME of diluted pesticides formulations (5 μg L⁻¹ Linr and Tebf and 9 μg L⁻¹ Acet and Imid) with tap water (a) before and (b) after standard addition of 5 μg L⁻¹ of pure pesticides.

the sample matrix effect. The relative recoveries of the studied pesticides (Table 3) in the tap water spiked with pure pesticides were in the range from 94 to 105%. The relative recoveries of the targeted pesticides from the wastewater are in the range from 89 to 123% indicated the presence of matrix effect. The similar effect has been reported [10,31] and it was suggested that for quantification in complex samples the standard addition method should be applied.

For the standard addition method, equal volumes of the samples (diluted pesticides formulation) are taken, and separately spiked with known amounts of pure pesticides (5, 10 and 15 μg L⁻¹) and diluted to the 10 mL with tap water. IL-VALLME was performed under optimal conditions. Fig. 5 shows HPLC chromatograms obtained after IL-VALLME of diluted pesticides formulations (5 μg L⁻¹ Linr and Tebf and 9 μg L⁻¹ Acet and Imid) with tap water (a) before and (b) after standard addition of 5 μg L⁻¹ of pure pesticides. The pesticides concentration, which was determined by the method of standard addition, were: 4.77, 5.19, 9.21 and 9.39 μg L⁻¹ for Linr, Tebf, Imid and Acet, respectively. These results indicate that developed IL-VALLME method is appropriate for the determination of the targeted pesticides in manufacturing wastewater.

4. Conclusions

In this work the first application of the binary component IL-VALLME with IL as the extractants for the simultaneous determination of the four pesticides in the manufacturing wastewater samples was reported.

The targeted pesticides belong to different chemical groups and possess $log\ P_{o/w}$ from 0.46 to 4.38. The effect of IL cation and anion structure, volume of IL and aqueous sample, pH and ionic strength, extraction and centrifugation time, on the extraction efficiency and enrichment factor, were investigated.

The highest extraction efficiency and enrichment factor were obtained applying $[C_6MIM][(CF_3SO_2)_2N]$ as extractant for all targeted pesticides. It was shown that due to the unique characteristic of the applied IL, such as, polarity and hydrophobicity, it is possible to perform almost complete extraction of the low polar pesticides ($log\ P_{o/w} > 3$) and to significantly enhance extraction of the more polar compounds ($log\ P_{o/w} \leq 1.5$). The optimized extraction parameters of the proposed IL-VALLME method are the following: the aqueous sample volume of 10 mL with addition of 0.58 g NaCl, 40 μL of $[C_6MIM][(CF_3SO_2)_2N]$ as extractant, 2 min extraction under the vigorous mixing applying the test tube shaker Vortex (2500 rpm), and separation of the phases by centrifugation for 2 min at 1000 rpm. The method was validated, good linearity, selectivity and reproducibility were obtained for the studied pesticides. Determined LOD values are 1.8, 2.3, 4.8 and 8.6 μg L⁻¹ for Tebf, Linr, Acet and Imid, respectively. These values are lower than the maximum acceptable concentration of the pesticides in manufacturing wastewater before discharged into sewage or aquatic environment (50 μg L⁻¹).

The proposed method was applied for determination of the pesticides in wastewater sample from the pesticides manufacturing. To avoid matrix effect, the standard addition method should be applied for

determination of the pesticides in the manufacturing wastewater.

One should emphasises the following benefits of this method: rapidness, simplicity, easy of the operation, low consumption of IL, simultaneous extraction of the low polar and more polar compounds, direct injection in HPLC, and the environmentally friendly aspect of the method. This work shows that IL-VALLME technique can be treated as a promising alternative to the other extraction techniques as the sample pre-treatment before HPLC determination of the pesticides in manufacturing wastewater samples.

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