SAMPLE PREPARATION TECHNIQUES
BASED ON EXTRACTION FOR ANALYSIS
OF PESTICIDES IN FOOD SAMPLES

Tatjana M. Trtić-Petrović,
Ksenija Kumrić, Jelena Đorđević, Aleksandra Dimitrijević
Laboratory of Physics
„Vinča” Institute of Nuclear Sciences
University of Belgrade, Belgrade, Serbia
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   • Results of optimization of extraction parameters
   • Method validation
   • Analysis of the targeted pesticides in fruit juices samples
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   • Results of optimization of extraction parameters
   • Method validation
   • Analysis of the targeted pesticides in fruit juices samples
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Introduction

• Pesticides are heterogeneous and numerous chemical or biological compounds that are used for the control of different sort of pests.
• More than 1200 compounds have been registered as pesticides.
• Agricultural production currently, and increasingly, depends on the use of pesticides.
• These compounds and the products of their degradation or metabolism may spread through environment and frequently contaminate surface and ground waters, soil, agricultural products and food.
• Several international organizations have established maximum residue limits (MRLs) for pesticides (EC Regulation No. 396/2005, Off. J. Eur. Commun. L70, 1-16; U.S. Department of Agriculture, Foreign Agricultural Service, Maximum Residue Limit Database)
Introduction

Phases in analysis of pesticides

In order to control and monitor pesticides and the products of their degradation, analytical methodologies must identify and quantify very low concentration of the pesticides in very complex samples.

Sample preparation step
  • Separation of analyte from matrix
  • Preconcentration

Determination of pesticides by GC, HPLC, with different detection system

Solid phase extraction

Liquid-liquid microextraction
Aim

The aim of this research is investigation of two sample preparation methods based on liquid/liquid microextraction for determination of the selected pesticides in fruit juices:

1. Two-phase membrane extraction in a single hollow fibre,
2. Ionic liquid based liquid-liquid microextraction
### Selected pesticides

<table>
<thead>
<tr>
<th>Pesticide (shortcut)</th>
<th>Chemical group (Activity)</th>
<th>Chemical structure</th>
<th>Partition coef. (oct/aq) logP* pH 2-8.5</th>
<th>Fruit / fruit juice</th>
<th>Average** /maximal concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidaclopride (IMI)</td>
<td>Neonikotinoid (insekticid)</td>
<td><img src="https://example.com/structure" alt="Imidaclopride structure" /></td>
<td>0.46</td>
<td>Grapes</td>
<td>72 / 2300</td>
</tr>
<tr>
<td>Acetamiprid (ACE)</td>
<td>Neonikotinoid (insekticid)</td>
<td><img src="https://example.com/structure" alt="Acetamiprid structure" /></td>
<td>1.55</td>
<td>Strawberries Apple juice</td>
<td>12 / 670 4 / 25</td>
</tr>
<tr>
<td>Carbendazime (CAR)</td>
<td>Benzimidazole (fungicide)</td>
<td><img src="https://example.com/structure" alt="Carbendazime structure" /></td>
<td>-0.16 - 1.52</td>
<td>Strawberries Apple juice Blueberries</td>
<td>29.9 / 930 4 / 25 2 / 1500</td>
</tr>
<tr>
<td>Simazine (SIM)</td>
<td>Triazine (herbicide)</td>
<td><img src="https://example.com/structure" alt="Simazine structure" /></td>
<td>2.26</td>
<td>Blueberries</td>
<td>0.2 / 120</td>
</tr>
<tr>
<td>Linuron (LIN)</td>
<td>Fenilurea (herbicide)</td>
<td><img src="https://example.com/structure" alt="Linuron structure" /></td>
<td>3.12</td>
<td>Carrot</td>
<td>18.7 / 330</td>
</tr>
<tr>
<td>Tebufenozid (TEB)</td>
<td>Diacilhidrazin (insekticid)</td>
<td><img src="https://example.com/structure" alt="Tebufenozid structure" /></td>
<td>4.38</td>
<td>Blueberries</td>
<td>5.5 / 1000</td>
</tr>
</tbody>
</table>

* determined by ACD/Labs PhysChem  
Two-phase membrane extraction in a single HF

Parameters influencing extraction:
- Organic phase
- Extraction time
- Sample volume

Schematic of two-phase membrane extraction

Polypropylene HF (Accurel)
Inner diameter 280 µm
Wall thickness 50 µm
Effective length 400 mm
Porosity 28%
Volume of org. ph. 30 µL

Experimental set-up

Donor: Aqueous/fruit juice sample spiked with pesticides
Effect of different extractants on the extraction efficiency of the pesticides from the aqueous sample

Extraction conditions:
- Donor: 50 cm$^3$ water
- $C_{\text{pesticide}} = 0.1$ mg dm$^{-3}$
- Extractants: DHE - dihexyl ether, TOPO - tri-n-octylphosphine oxide, TBP - tri-n-butyl phosphate, DEHPA - di(2-ethylhexyl)phosphoric acid
- Extraction time: 120 min
- Shaking rate: 100 rpm

Optimal organic phase: 10% TOPO + 10% TBP in DHE
Results

Effect of the extraction time

![Graph showing the effect of extraction time on peak area for Tebufenozid and Linuron.]

- Extraction conditions: donor – 50 mL water (pH 5.8) spiked at 0.1 mg dm\(^{-3}\) of each pesticide,
- Extractants: 10% TOPO and 10% TBP in DHE, shaking rate: 100 rpm.

Optimal extraction time: 4 h

Effect of the sample volume

![Graph showing the effect of sample volume on peak area for TEB, LIN, and SIM.]

- Extraction conditions: donor – 50 - 1000 mL water (pH 5.8) spiked at 0.1 mg dm\(^{-3}\) of each pesticide,
- Extractants: 10% TOPO and 10% TBP in DHE, shaking rate: 100 rpm. Extraction time: 4 h.

Optimal volume of aqueous phase 500mL and organic phase 30 µL
Results

The selected parameters of the calibration curves

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Concentration range (mg dm(^{-3}))</th>
<th>(r^2)</th>
<th>LOD (µg dm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIM</td>
<td>0.05 - 1.0</td>
<td>0.9985</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>0.9975</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>TBF</td>
<td>0.001 - 0.25</td>
<td>0.9997</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>0.9991</td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>LIN</td>
<td>0.001 - 0.25</td>
<td>0.9987</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>0.9986</td>
<td></td>
<td>6.8</td>
</tr>
</tbody>
</table>

- The extraction procedure is cheap, selective, and simple;
- no additional sample treatment is needed before the application of the two-phase HF-LPME
- low volume of organic solution
- good linearity, low detection limits for the pesticides with \(\log P > 2\)
Ionic liquid (IL)

- ILs are salt
- ILs content of organic cations and inorganic or organic anions
- ILs are liquid at RT
- The properties of ILs can be tunable depend on the cation and the anion

**Cations:**
A: 1-alkyl-3-methyl-imidazolium  
B: 1-alkyl-pyridinium  
C: 1,1-dialkyl-pyrrolidinium  
D: tetraalkyl-ammonium  
E: tetraalkyl-phosphonium

**Anions**
F: bis(trifluoromethylsulfonyl)imide  
G: trifluoromethylsulfonate  
H: dicyanamide  
I: alkyl sulphate  
J: tosilate
Experimental part

Ionic liquid vortex assisted based liquid-liquid microextraction (IL-VALLME)

**Ionic liquids**: 1-heksil-3-metilimidazol bis(trifluorometilsulfonil)imid
\[ [C_6\text{MIM}]\text{[(CF}_3\text{SO}_2)_2\text{N}] \]
- Density 1.33 g ml\(^{-1}\)
- Solubility in water 0.34 g dm\(^{-1}\)

**Experimental set-up**:
- Aqueous solution / fruit juice with pesticides
- Addition of IL
- Shaken using a vortex agitator
- IL was separated from aqueous solution by centrifugation at 1000 rpm
- Determination of pesticides in IL using HPLC/DAD

**Parameters influencing extraction**:
- Volume of IL
- Sample volume
- Extraction time
Optimization of the extraction conditions

Partition coefficient

<table>
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<tr>
<th>Pesticide</th>
<th>logP (IL/aq)</th>
<th>logP (oct/aq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>1.61</td>
<td>0.46</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>1.65</td>
<td>1.55</td>
</tr>
<tr>
<td>Carbendazime</td>
<td>1.64</td>
<td>0.72</td>
</tr>
<tr>
<td>Simazine</td>
<td>1.60</td>
<td>2.26</td>
</tr>
<tr>
<td>Linuron</td>
<td>3.5</td>
<td>3.12</td>
</tr>
<tr>
<td>Tebufenozid</td>
<td>4.13</td>
<td>4.38</td>
</tr>
</tbody>
</table>

Effect of volume of IL on removal efficiency and enrichment factor of imidacloprid

Extraction conditions: sample 5 mL water (pH 5.8) spiked at 0.1 mg dm\(^{-3}\) of each pesticide, shaking rate: 100 rpm, extraction time: 60 min.

Optimal volume of IL: 40 µL
Optimization of the extraction conditions

Effect of the extraction time

Optimal extraction time: 2 min

Effect of the volume ratio of sample and IL

Optimal ratio of $V_{aq}/V_{IL}$: 200
The selected parameters of calibration curves of the pesticides from water and blueberry juice

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>$r^2$</th>
<th>LOD (µg dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>0.9985</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>0.9964</td>
<td>9.5</td>
</tr>
<tr>
<td>Acetamipride</td>
<td>0.9981</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>0.9963</td>
<td>6.4</td>
</tr>
<tr>
<td>Carbendazole</td>
<td>0.9993</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>0.9988</td>
<td>4.3</td>
</tr>
<tr>
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<td>0.9988</td>
<td>5.4</td>
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<td>Tebufenozid</td>
<td>0.9998</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>0.9986</td>
<td>3.0</td>
</tr>
</tbody>
</table>

(Concentration range 0.005 – 0.25 mg dm$^{-3}$)

**Optimal extraction conditions:**
- volume of sample: 10 mL
- IL: 0.04 mL [C$_6$MIM][CF$_3$SO$_2$]$_2$N
- extraction time 2 min
- shaking rate 2500rpm (Vortex)
- separation: centrifugation for 2 min at 1000 rpm

**Chromatogram of “real sample”**

\[ C_{IMI} = 2.3 \text{ µg dm}^{-3} \]
\[ C_{TBF} = 1.8 \text{ µg dm}^{-3} \]

- Method is fast and simply,
- low consumption of IL,
- simultaneous extraction of the low polar and more polar compounds,
- direct injection in HPLC.
Concluding remark

This work shows that the two-phase membrane extraction and ionic liquid based microextraction procedures can be considered as a sound alternative to other extraction techniques as the sample pre-treatment before HPLC determination of pesticides in fruit juices.

Thank you for your attention!