Article

Homogeneous Liquid–Liquid Microextraction for Determination of Organophosphorus Pesticides in Environmental Water Samples Prior to Gas Chromatography-Flame Photometric Detection

Sana Berijani1,*, Mirhanif Sadigh2, and Elham Pournamdari3

1Department of Applied Chemistry, Faculty of Science, Islamic Azad University, South Tehran Branch, Tehran, Iran, 2Department of Civil Engineering, University of Science and Culture, Tehran, Iran, and 3Department of Chemistry, College of Science, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

*Author to whom correspondence should be addressed. Email: berijani@gmail.com

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Abstract

In this study, homogeneous liquid–liquid microextraction (HLLME) was developed for preconcentration and extraction of 15 organophosphorus pesticides (OPPs) from water samples coupling with gas chromatography followed by a flame photometric detector (HLLME-GC-FPD). In this method, OPPs were extracted by the homogeneous phase in a ternary solvent system (water/acetic acid/chloroform). The homogeneous solution was excluded by the addition of sodium hydroxide as a phase separator reagent and a cloudy solution was formed. After centrifugation (3 min at 5,000 rpm), the fine particles of extraction solvent (chloroform) were sedimented at the bottom of the conical test tube (10.0 ± 0.5 µL). Furthermore, 0.5 µL of the sedimented phase was injected into the GC for separation and determination of OPPs. Optimal results were obtained under the following conditions: volume of the extracting solvent (chloroform), 53 µL; volume of the consolute solvent (acetic acid), 0.76 mL and concentration of sodium hydroxide, 40% (w/v). Under the optimum conditions, the enrichment factors of (260–665), the extraction percent of 75.8–104%, the dynamic linear range of 0.03–300 µg L−1 and the limits of detection of 0.004–0.03 µg L−1 were obtained for the OPPs. This method was successfully applied for the extraction and determination of the OPPs in environmental water samples.

Introduction

Organophosphorus pesticides (OPPs) represent one of the most important classes of cholinesterase inhibitors (1). They are very toxic when absorbed by human organisms because of acetyl-cholinesterase deactivation. In addition, OPPs are known to reduce the activity of neurotransmitters and hence to cause irreversible effects on the nervous system (2). They are also important causes of morbidity and mortality following intentional self-poisoning or in the case of occupational or environmental exposure (3). Determination of OPPs in water is usually carried out by methods involving gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography (HPLC) (4–9). Monitoring environmental pollutants at an ultra-trace level needs an effective sample preconcentration step (10) and due to the low OPP concentration and complex matrices, the sample is prepared by liquid–liquid extraction (LLE) and also by solid-phase extraction (SPE) (11, 12). SPE is a solvent-free process, developed by Anrthur and Pawliszyn (13), that features simultaneous extraction and preconcentration of analytes directly from an aqueous and gas sample or from the headspace of aqueous and solid samples (14, 15). This technique is fast, portable, easy to use and is applied for determination of OPPs in water samples (6, 16, 17). Liquid-phase microextraction (LPME) and also single-drop microextraction (SDME) were reported in 1997 (18, 19). SDME was used for determination of OPPs in water samples (20, 21). Cloud point extraction (CPE), a kind of LLE technique, has been used in different fields of analytical chemistry, mainly those focusing on separation and preconcentration based on cloud point
procedures (22–24). This technique has also been used for extraction of OPPs (25, 26). Dispersive liquid–liquid microextraction (DLLME) was developed and reported as a simple and rapid technique for determination of different compounds in water and was also performed for determination of OPPs in water (27–31). Ultrasound-assisted emulsification microextraction is another sample pretreatment method, which has been introduced by Regeiro in 2008, and has been applied for determination of different pollutants (32–35). The homogeneous liquid–liquid microextraction (HLLME) method, which extracts the desired analyte existing in the homogeneous aqueous solution into the water-immiscible sediment phase, is based on the phenomenon of phase separation (36). In this method, the initial condition is a homogeneous solution and there is no interface between the water phase and the extraction solvent phase. Therefore, it has the advantage of extremely fast extraction speed due to the absence of obstacles from the surface contact between the aqueous phase and the organic phase during the extraction procedure. This method was mainly studied as a high-powered preconcentration method for the separation of the desired component or instrumental analysis (37–41). The ternary component solvent system and the perfluorinated surfactant system are the two usual modes of homogeneous LLE (42). The goal of this study was to assess the technique suitability for the detection of 15 OPPs in the water samples. The analytes were monitored by gas chromatography combined with flame photometric detector (GC-FPD). Finally, this recommended method was employed to investigate the levels of the target species in environmental water samples.

Experimental

Instrumentation and reagents

All OPPs (dichlorovos, mevinphose, phorate, diazinon, disolfotane, methylparathion, sumithion, chloropyrifos, malathion, fenitrothion, proflufenphos, ethion, phosalone, azinophospho-methyl, co-rad) were purchased from Poly Science Company. Chloroform, acetic acid, triphenyl phosphate (as internal standard) and sodium hydroxide, so the water-immiscible sedimented phase, consisting of hydrophobic solvent, is separated from the aqueous solution. To obtain a good recovery, the effect of some parameters was examined and optimum conditions were selected. To study the explained parameters, extraction recovery and enrichment factor have been used (Equations (1) and (2)).

\[ R\% = \frac{C_{\text{sed}} \times V_{\text{sed}}}{C_0 \times V_{\text{aq}}} \times 100 \]  
(1)

\[ \text{EF} = \frac{C_{\text{sed}}}{C_0} \]  
(2)

where \( R\% \) is the extraction recovery, EF defines as enrichment factor, \( C_{\text{sed}} \) and \( C_0 \) refer to the concentration of analytes in the sediment phase and the initial aqueous phase, \( V_{\text{sed}} \) and \( V_{\text{aq}} \) are the volume of the sediment phase and the aqueous phase, respectively. All the mentioned parameters are known except \( C_{\text{sed}} \). Calculation of \( C_{\text{sed}} \) was done by direct injection of OPPs standard solutions with different concentrations in the range of 0.5–2.5 mg L\(^{-1}\).

Discussion

Selection of volume of the extracting solvent

In this ternary solvent system, chloroform acts as an extracting solvent. To examine the effect of the volume of chloroform, solutions containing different volumes of chloroform were examined with the same HLLME procedure. Hence, 0.76 mL acetic acid containing 45, 49, 53, and 60 µL chloroform were tested. Figures 2, 3, and 4 show the curves of volume of sediment phase, extraction recovery and enrichment factor versus the volume of chloroform. As shown in Figure 2, by increasing the volume of chloroform from 45 to 60 µL, the volume of the sediment phase increases from 4 to 18 µL. Figure 3 indicates that by increasing the volume of chloroform, the extraction recovery increases up to 53 and it will remain constant from 53 to 60 µL.

Results

In ternary solvent system (water/acetic acid/chloroform), acetic acid molecules can solvate chloroform molecules and a homogeneous solution will be formed. When the solvation effect of the acetic acid is excluded by addition of sodium hydroxide, acetic acid converts to acetate ions due to the acid–base reaction of acetic acid and sodium hydroxide, so the water-immiscible sedimented phase, consisting of hydrophobic solvent, is separated from the aqueous solution. To obtain a good recovery, the effect of some parameters was examined and optimum conditions were selected. To study the explained parameters, extraction recovery and enrichment factor have been used (Equations (1) and (2)).

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(1)

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of chloroform. Figure 4 shows that increasing the volume of the chloroform and sediment phase leads to decreasing of enrichment factors. It should be noticed that by using the volumes <40 µL of chloroform, no sediment phase is formed and the volumes >60 µL cause no homogeneous solution. Therefore, 53 µL was selected as the optimum volume of chloroform.

**Selection of phase separator reagent concentration**

In HLLME after formation of homogeneous solution according to the components of the solution, a reagent is spiked in order to separate the organic and aqueous solutions. In the selected system, homogeneous solution of chloroform in water in the presence of acetic acid was created in which the chloroform molecules are solvated by acetic acid molecules and dissolved in water. Sodium hydroxide acts as a reagent, which excludes the homogeneous solution and makes two phases.

Figure 1. Scheme of HLLME in this research.

Figure 2. Effect of the volume of chloroform on the volume of sediment phase.

Figure 3. Effect of the volume of chloroform on the recovery of OPPs.

Figure 4. Effect of the volume of chloroform on the enrichment factor of OPPs.
Sodium hydroxide enters an acid–base reaction with acetic acid, and sodium acetate is formed in the solution. In this way, chloroform which contains the analytes separates from aqueous solution and the homogeneous solution converts to a cloudy solution. After centrifugation, a sediment phase was formed and 0.5 µL of this phase was used for analysis. Different concentrations of sodium hydroxide 25, 30, 35 and 40 (% w/v) were prepared and tested in the experiment. The addition of ionic strength promotes the transport of analytes into the organic phase, so the extraction efficiency increases while increasing the NaOH up to 40% (w/v). However, an increase in the sediment phase volume and dilution effect of chloroform leads to a decrease in the preconcentration of OPPs (Figs 5–7). Finally, sodium hydroxide 40 (%w/v) was selected for the analysis. It is recognizable that the use of higher concentration of sodium hydroxide was not possible because of the heat produced in acid–base reaction that may evaporate the chloroform, and also the alkaline medium may destroy some of the OPPs.

Effect of extraction time
Extraction time is one of the major parameters affecting the extraction efficiency. In HLLME, the profile of extraction time was studied by monitoring the variation of the relative peak area of the analyte with the time of cloudy state before starting centrifugation. The effect of time was examined in the range of 0–60 min under optimum extraction conditions (Figure 8). According to the curves,
time has no effect on extraction efficiency. It is revealed that after formation of cloudy solution, the surface area between the extraction solvent and the aqueous phase is infinitely large. Thereby, transition of analytes from the aqueous phase (sample) to the extraction solvent is fast. Subsequently, equilibrium state is achieved quickly because of the short time of the extraction. In this extraction method, the only time-consuming step is centrifugation, which is ∼3 min.

**Table I.** Quantitative results of HLLME-GC-FPD of OPPs from the water sample

<table>
<thead>
<tr>
<th>OPPs</th>
<th>RSD %a (n = 5)</th>
<th>RSD %b (n = 5)</th>
<th>EF</th>
<th>LDR (µg L⁻¹)</th>
<th>r²c</th>
<th>r²d</th>
<th>LOD (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorovos</td>
<td>1.6</td>
<td>2.4</td>
<td>317</td>
<td>0.05–300</td>
<td>0.998</td>
<td>0.996</td>
<td>0.02</td>
</tr>
<tr>
<td>Mevinphose</td>
<td>3.0</td>
<td>6.3</td>
<td>274</td>
<td>0.05–300</td>
<td>0.998</td>
<td>0.994</td>
<td>0.02</td>
</tr>
<tr>
<td>Phorate</td>
<td>4.0</td>
<td>7.1</td>
<td>327</td>
<td>0.02–300</td>
<td>0.999</td>
<td>0.994</td>
<td>0.005</td>
</tr>
<tr>
<td>Diazinon</td>
<td>1.9</td>
<td>5.2</td>
<td>325</td>
<td>0.02–300</td>
<td>0.999</td>
<td>0.998</td>
<td>0.01</td>
</tr>
<tr>
<td>Disolfotane</td>
<td>5.4</td>
<td>8.8</td>
<td>260</td>
<td>0.03–300</td>
<td>0.999</td>
<td>0.996</td>
<td>0.01</td>
</tr>
<tr>
<td>Methylparathion</td>
<td>5.7</td>
<td>8.2</td>
<td>665</td>
<td>0.03–300</td>
<td>0.998</td>
<td>0.995</td>
<td>0.01</td>
</tr>
<tr>
<td>Sumithion</td>
<td>3.6</td>
<td>6.6</td>
<td>369</td>
<td>0.03–300</td>
<td>0.998</td>
<td>0.997</td>
<td>0.01</td>
</tr>
<tr>
<td>Chloropyrifos</td>
<td>1.8</td>
<td>5.2</td>
<td>344</td>
<td>0.03–300</td>
<td>0.991</td>
<td>0.994</td>
<td>0.01</td>
</tr>
<tr>
<td>Malathion</td>
<td>6.3</td>
<td>7.4</td>
<td>455</td>
<td>0.03–300</td>
<td>0.998</td>
<td>0.993</td>
<td>0.01</td>
</tr>
<tr>
<td>Fenthion</td>
<td>3.0</td>
<td>5.6</td>
<td>324</td>
<td>0.03–300</td>
<td>0.999</td>
<td>0.993</td>
<td>0.01</td>
</tr>
<tr>
<td>Profenphos</td>
<td>3.4</td>
<td>4.6</td>
<td>658</td>
<td>0.03–300</td>
<td>0.999</td>
<td>0.992</td>
<td>0.01</td>
</tr>
<tr>
<td>Ethion</td>
<td>4.4</td>
<td>5.6</td>
<td>315</td>
<td>0.02–300</td>
<td>0.998</td>
<td>0.998</td>
<td>0.005</td>
</tr>
<tr>
<td>Phosalone</td>
<td>4.2</td>
<td>3.9</td>
<td>478</td>
<td>0.03–300</td>
<td>0.999</td>
<td>0.993</td>
<td>0.01</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td>8.5</td>
<td>10.8</td>
<td>562</td>
<td>0.05–300</td>
<td>0.996</td>
<td>0.994</td>
<td>0.02</td>
</tr>
<tr>
<td>Co-Ral</td>
<td>1.1</td>
<td>3.8</td>
<td>394</td>
<td>0.1–300</td>
<td>0.998</td>
<td>0.993</td>
<td>0.04</td>
</tr>
</tbody>
</table>

EF, enrichment factor; LOD, limit of detection for a S/N = 3.
aRSD% by using the internal standard at a concentration of 2 µg L⁻¹ of each OPP.
bRSD% without using the internal standard.
cCalculated by using the internal standard.
dWithout using the internal standard.

**Table II.** Comparison of HLLME with DLLME, SDME and SPME for determination of OPPs in water

<table>
<thead>
<tr>
<th>Methods</th>
<th>LOD (µg L⁻¹)</th>
<th>LDR (µg L⁻¹)</th>
<th>RSD (%)</th>
<th>Sample volume (mL)</th>
<th>Extraction time (min)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPME-GC-FPD</td>
<td>0.03–0.4</td>
<td>1–50</td>
<td>5–8</td>
<td>3</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>SPME-GC-NPD</td>
<td>0.02–0.04</td>
<td>0.1–10</td>
<td>7–17</td>
<td>3</td>
<td>60</td>
<td>9</td>
</tr>
<tr>
<td>SDME-GC-MS</td>
<td>0.01–0.07</td>
<td>0.5–100</td>
<td>8.5–15</td>
<td>5</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>SDME-GC-FPD</td>
<td>0.002–0.02</td>
<td>0.01–100</td>
<td>7.9–13</td>
<td>5</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>DLLME-GC-FPD</td>
<td>0.003–0.02</td>
<td>0.01–100</td>
<td>4.6–6.5</td>
<td>5</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>HLLME-GC-FPD</td>
<td>0.004–0.03</td>
<td>0.03–300</td>
<td>1.1–7.5</td>
<td>7</td>
<td>5</td>
<td>Represented method</td>
</tr>
</tbody>
</table>

**Table III.** Results of real sample analysis

<table>
<thead>
<tr>
<th>OPPs</th>
<th>Concentration in real sample (SD)a (µg L⁻¹)</th>
<th>Added concentration (µg L⁻¹)</th>
<th>Founded concentration (SD) (µg L⁻¹)</th>
<th>Relative recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorovos</td>
<td>ndb</td>
<td>0.50</td>
<td>0.379 ± 0.010</td>
<td>75.8 ± 2.0</td>
</tr>
<tr>
<td>Mevinphose</td>
<td>nd</td>
<td>0.50</td>
<td>0.503 ± 0.078</td>
<td>106.0 ± 15.6</td>
</tr>
<tr>
<td>Phorate</td>
<td>nd</td>
<td>0.50</td>
<td>0.486 ± 0.002</td>
<td>97.2 ± 0.4</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.113 ± 0.005</td>
<td>0.50</td>
<td>0.563 ± 0.018</td>
<td>90.0 ± 2.8</td>
</tr>
<tr>
<td>Disolfotane</td>
<td>nd</td>
<td>0.50</td>
<td>0.519 ± 0.003</td>
<td>103.8 ± 0.6</td>
</tr>
<tr>
<td>Methylparathion</td>
<td>nd</td>
<td>0.50</td>
<td>0.465 ± 0.013</td>
<td>93.0 ± 2.6</td>
</tr>
<tr>
<td>Sumithion</td>
<td>nd</td>
<td>0.50</td>
<td>0.470 ± 0.009</td>
<td>94.0 ± 1.8</td>
</tr>
<tr>
<td>Chloropyrifos</td>
<td>nd</td>
<td>0.50</td>
<td>0.524 ± 0.011</td>
<td>104.0 ± 2.18</td>
</tr>
<tr>
<td>Malathion</td>
<td>nd</td>
<td>0.50</td>
<td>0.493 ± 0.002</td>
<td>98.6 ± 0.4</td>
</tr>
<tr>
<td>Fenthion</td>
<td>nd</td>
<td>0.50</td>
<td>0.490 ± 0.011</td>
<td>98.0 ± 2.2</td>
</tr>
<tr>
<td>Profenphos</td>
<td>nd</td>
<td>0.50</td>
<td>0.388 ± 0.009</td>
<td>77.6 ± 1.8</td>
</tr>
<tr>
<td>Ethion</td>
<td>0.042 ± 0.007</td>
<td>0.50</td>
<td>0.563 ± 0.004</td>
<td>104.2 ± 0.7</td>
</tr>
<tr>
<td>Phosalone</td>
<td>nd</td>
<td>0.50</td>
<td>0.500 ± 0.045</td>
<td>100.0 ± 9.0</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td>nd</td>
<td>0.50</td>
<td>0.515 ± 0.032</td>
<td>103.0 ± 6.4</td>
</tr>
<tr>
<td>Co-Ral</td>
<td>nd</td>
<td>0.50</td>
<td>0.516 ± 0.046</td>
<td>103.2 ± 9.2</td>
</tr>
</tbody>
</table>

SD, standard deviation; nd, not detected.
Table II indicates the LOD, linear dynamic range (LDR), RSD, extraction time and sample volume in the SPME, SDME, DLLME and HLLME (represented method) for extraction and determination of OPPs from the water sample. The comparison of the results shows that the extraction time in HLLME is very short, ∼5 min, and also extraction equilibrium is achieved very quickly. Otherwise, the extraction time for SPME and SDME ranges from 15 to 60 min. The RSDs are low (1.1–7.5%) for HLLME, which are probably because of quick achievement of equilibrium (Figure 8). In comparison with other extraction methods, HLLME has low LODs (0.004–0.03 µg L⁻¹) and wide linear range (0.03–300 µg L⁻¹) and can be applied for determination of OPPs successfully with satisfactory results as other extraction and preconcentration techniques.

Conclusion
This article has outlined the successful development and application of a method based on the HLLME technique combined with the GC-FPD for the analysis of OPPs in water samples. The designed method is concluded to be precise, reproducible and linear over a broad range with sufficient selectivity. This analytical technique offers numerous advantages such as simplicity, low cost, ease of operation and high enrichment factors. In addition, the technique requires only a small volume of organic solvent, being therefore an environmentally friendly approach. The results show that performance of this procedure in the extraction of OPPs from different water samples with various matrices was excellent. Subsequently, this method can be used routinely for screening purposes.

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Determination of Organophosphorus Pesticides in Environmental Water Samples


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