Comparison of Solid-Phase Extraction and Micro-Solid-Phase Extraction for Liquid Chromatography/Mass Spectrometry Analysis of Pesticides in Water Samples

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Our recent on-line solid-phase extraction (SPE) device for micro-liquid chromatography, known as micro-solid-phase extraction (μSPE), was compared with traditional SPE for the analysis, from aqueous samples, of 4 pesticides belonging to different classes. Two different kinds of adsorbents, C18 and graphitized carbon black, were tested. A 2-stage ion trap mass spectrometer, equipped with homemade microflow electrospray ion (ESI) source, was used. Detection limits with a signal-to-noise ratio of 3:1 for both extraction methods were in the range of 0.1 μg/L for all compounds. However, better recoveries were obtained when μSPE traps were used.

The total amount of pesticides produced for both agricultural and industrial purposes is increasing. As a consequence of complex transport processes, these compounds are present worldwide in all environmental compartments. Gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) have always been the techniques of choice for environmental monitoring (1–4). However, many pesticides and their metabolites are polar and thermolabile, and must be detected at very low concentrations. These issues can only be considered with the help of liquid chromatography (LC) coupled with MS. Atmospheric pressure ionization (API) techniques, like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), have significantly improved the capabilities of LC in terms of selectivity and sensitivity and therefore widened the field of LC applications in environmental analysis. In particular, during the last few years, methods using both APCI and ESI have been developed for many classes of pesticides, including triazines, phenylureas, organophosphates, carbamates, chlorophenoxy acids, and sulfonylureas (5–18).

The European Union Drinking Water Directive (19) states that individual pesticides must be quantified at or below the 0.1 μg/L in drinking water. To meet such requirements, a trace-enrichment step is needed, because conventional LC/MS technology alone is not capable of reaching these low levels by direct sample injection. Solid-phase extraction (SPE) using C18 or carbon cartridges has been traditionally used to analyze pesticides in water samples (20–22). However, SPE can lead to sample losses due to irreversible adsorption onto the cartridge walls or during the solvent evaporation step, especially for volatile compounds.

Our research group recently developed an on-line SPE device for micro-LC with the advantage of reducing time, solvent consumption, and sample handling (23). The new device is based on the retention capability of a short-slurry packed LC column that acts as a micro trap. After the sampling step, the trap is connected to a reversed-phase capillary column to create a continuous on-line desorbing and analyzing apparatus. The trap can be easily replaced and re-used when needed. The aim of using a short column in the trapping step is to maximize sampling flow rate, minimizing the extraction time. Furthermore, the miniaturization of chromatographic column parameters brings advantages in terms of solvents and sample consumption and may enhance sensitivity when coupled to a mass spectrometer.

In this work, the micro-solid-phase extraction (μSPE) approach was compared with traditional SPE for the extraction and concentration of 5 pesticides used in flower treatments of an agricultural area of central Italy. Two kinds of absorbent materials, C18 and graphitized carbon black, were also evaluated. An ion trap mass spectrometer equipped with an ESI source was used as a detector. The modification of ESI interface for microflow rates further increases sensitivity (24). In fact, the instrument response is strictly dependent on sample concentration. Both SPE techniques were compared for recovery, reproducibility, sample handling, time, and solvent consumption.

Experimental

Reagents and Chemicals

(a) Solvents.—LC grade (J.T. Baker, Phillipsburg, NJ); de-gassed before use.

(b) Pesticides.—Riedel-de Haen (Hannover, Germany). See Table 1.

(c) Distilled water.—Milli-Q water purification system (Millipore Corp., Bedford, MA).
**Table 1. Chemical properties of selected pesticides**

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>MW</th>
<th>Class</th>
<th>CAS-RN</th>
<th>Solubility in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>162</td>
<td>Carbamoyloxime</td>
<td>16752-77-5</td>
<td>58 g/L at 25°C</td>
</tr>
<tr>
<td>Benomyl</td>
<td>290</td>
<td>Benzimidazole</td>
<td>17804-35-2</td>
<td>4 mg/L at 25°C</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>225</td>
<td>Carbamate</td>
<td>2032-65-7</td>
<td>27 mg/L at 20°C</td>
</tr>
<tr>
<td>Methidathion</td>
<td>302</td>
<td>Organophosphorus</td>
<td>950-37-8</td>
<td>250 mg/L at 20°C</td>
</tr>
</tbody>
</table>

(d) **Stock solution of the selected pesticides.**—At concentration of 1.6 ng/µL in methanol.

(e) **Stationary phase.**—(1) C18.—Reversed-phase 5 µm particle size (Phase Sep, Queensferry, UK). (2) Hypercarb.—With 7 µm particle size and Carbograph 1 (surface area, 100 m²/g); from Hypersil (Thermoquest Chromatography Supplies, Needham, MA) and from LARA (Laboratori Analitici di Ricerca Associati, Rome, Italy), respectively.

**Sampling**

Samples of well and irrigation canal water were collected near Pesaro (central Italy) and stored at 4°C in the dark. Before analysis, the irrigation water was filtered using 0.45 µm pore size Millipore membranes.

**Liquid Chromatography/Mass Spectrometry**

Separations were performed with a Spectra Series P2000 liquid chromatograph (Thermo Separation Products, San Jose, CA). During analysis, the LC solvent composition varied from 100% water to 100% acetonitrile in 25 min. A flow rate of 2 µL/min was obtained with a homemade splitter device placed between the pumping system and the injector (25). C18 column (15 cm long x 300 µm id) packed with 3 µm particles was kindly supplied by LC Packings ( Dionex Co., San Francisco, CA).

The column was connected to a ThermoFinnigan LCQ-DUO LC-ESI-MS system (ThermoQuest LC/MS Division, San Jose, CA), equipped with an ion trap analyzer for single-stage MS/MS analysis. The ESI source was modified to work with flow rates of a few µL/min (Figure 1). In particular, the sample tube (200 µm od x 100 µm id) was substituted by a fused silica capillary tubing (192 µm od x 50 µm id), which was heated on a direct flame and stretched to sharpen its tip. This homemade ESI capillary was positioned inside the ESI needle and placed very close to the inlet of the mass spectrometer to enhance the ion sampling efficiency. The mass spectrometer worked in positive ion mode and the micro-ESI interface voltages, optimized to achieve maximum signal-to-noise ratio, were 4.5 kV on the ESI needle and 10 V on the heated capillary. The source temperature was 170°C, and nitrogen was used to assist nebulization at a pressure of 3 atm. Ions were accumulated in the ion trap for 1000 ms, while 3 microscans were summed up.

For recovery studies, the concentrations of the analytes were calculated by measuring peak areas from extracted-ion current (EIC) profiles and comparing them with those obtained from stock solution injections.

**SPE System**

The SPE extraction was performed according to a well-known method described by Di Corcia and Marchetti (20). Extraction cartridges were filled with Carbograph 1 (250 mg) or C18 (500 mg). Before use, the cartridge was washed with 5 mL methylene chloride–methanol (80 + 20, v/v) followed by 2 mL methanol. The C18 cartridge was then rinsed with 15 mL water to eliminate any trace of

![Figure 1. Schematic view of homemade ESI source assembly.](image1)

![Figure 2. Scheme of µSPE system.](image2)
methanol, and the Carbograph 1 cartridge was rinsed with 15 mL of 10 g/L ascorbic acid–acidified water, pH 2.

Samples of distilled water, well water, and canal water (1.7 L) were spiked with 100 μL of the 1.6 ng/μL standard solution. Before sampling, a shaking step is necessary to minimize analyte deposition on the flask walls. The use of a Teflon flask is advisable as well. Each sample was forced to pass through the cartridge by vacuum from a water pump. After sampling, the flask and walls of the cartridge were rinsed with 10 mL distilled water to remove sample water drops. The cartridge was then dried by pulling air for 1 min. The pesticides were eluted in forward flush mode from the carbon cartridge with 1 mL methanol followed by 6 mL methylene chloride–methanol (80 + 20, v/v), and from the C18 cartridge with 5 mL methanol followed by 3 mL methylene chloride–methanol (80 + 20, v/v). After extraction, the sample was concentrated to a final volume of 100 μL under a gentle stream of nitrogen at room temperature. The entire process required at least 120 min. The sample was injected into the LC device by an injector equipped with a 60 nL internal loop, and analyzed.

μSPE System

We packed the μSPE traps using a slurry packing technique, which is the same as that used to prepare packed capillary columns (26). The slurry is a suspension of the stationary phase in acetonitrile mixed in a ratio of 1 mg stationary phase to 4 mL acetonitrile. The suspension was then shaken to make it homogeneous and positioned into a stainless steel reservoir. The trap consisted of 3 cm long PEEK tubing with 250 μm id (Figure 2), one side closed by a porous frit to keep the stationary phase back. The other side was connected to the reservoir and the slurry was forced into the trap by a pressure of 120 bar acetonitrile, using a Shimadzu LC-5A pump (Shimadzu Corp., Tokyo, Japan). The total packing time was 10–15 min. Then, 1 h water conditioning was necessary to adjust the packing bed. We used 2 types of stationary phases: C18 with 5 μm particle size and Hypercarb with 7 μm particle size. Hypercarb consists of a rigid silica core in which are fixed very small particles of graphitized carbon with specific surface area of 120 m²/g. Graphitized Carbograph 1, used in SPE methods, cannot be used because it is friable. In fact, it would suffer from extremely high pressures used in sampling and LC analysis procedures. The silica core of Hypercarb overcomes this problem and allows comparison of the 2 adsorbents which have a very similar specific surface area (Carbograph 1: 100 m²/g).

Results and Discussion

µESI Interface Performance Optimization

The optimization procedure was performed by injecting each pesticide in flow injection analysis (FIA) for preliminary adjustment. The flow rate was 2 μL/min, and the mobile phase composition was kept at 50% water and 50% acetonitrile. An injector with a 60 nL internal loop was used. In order to obtain the highest signal intensity, different parameters were optimized: spray voltage, position of the thinned capillary, pressure of the nebulizing gas, and heated capillary potential. Full scan mass spectra of the selected pesticides, obtained as described in the Experimental section, are reported in Table 2. Benomyl showed a complete fragmentation into the ESI source. Methomyl and methidathion exhibited prominent sod- dium adduct ions, while m/z 169 was the most abundant ion for methiocarb, due to the loss of the methyl isocyanate group (Me–N=C=O). The adducts are formed as a consequence of minimum salt amounts present in the organic solvents and glassware (9).

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>MW</th>
<th>[M + H]$^+$</th>
<th>Adducts</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>162</td>
<td>185[M+Na]$^+$ (100%)</td>
<td>88 (70%)</td>
<td></td>
</tr>
<tr>
<td>Benomyl</td>
<td>290</td>
<td>192 (100%)</td>
<td>160 (20%)</td>
<td></td>
</tr>
<tr>
<td>Methiocarb</td>
<td>225</td>
<td>226 (30%)</td>
<td>248[M + Na]$^+$ (60%)</td>
<td>169 (100%)</td>
</tr>
<tr>
<td>Methidathion</td>
<td>302</td>
<td>325[M+Na]$^+$ (100%)</td>
<td>145 (50%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Time-scheduled SIM conditions for LC–ESI–MS analysis of selected pesticides

<table>
<thead>
<tr>
<th>Time (min in LC traces)</th>
<th>Compound (m/z monitored)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 0 to 28</td>
<td>Methomyl (185)</td>
</tr>
<tr>
<td></td>
<td>Benomyl (192)</td>
</tr>
<tr>
<td>From 28</td>
<td>Methiocarb (169)</td>
</tr>
<tr>
<td></td>
<td>Methidathion (325)</td>
</tr>
</tbody>
</table>
For all the compounds, fragmentation increased when heated capillary potential was gradually increased to 50 V. All the chromatographic experiments were performed in selected-ion monitoring (SIM) mode, with the most abundant ion chosen from each pesticide (Table 3). For methomyl and methidathion, \([M + Na]^+\) was considered. Although some papers (9, 16) report that the use of sodium adduct ions could lead to irreproducible results, we decided to use them for their extremely high response. We tested the repeatability of the methods, obtaining acceptable values (see Instrumental Precision section). Although a sodium salt could be added to the mobile phase to avoid sodium concentration variability, we decided not to use it because it could increase the conductivity of the mobile phase and cause instability in the electrospray (18).

**Limits of Detection (LODs)**

The LODs (signal-to-noise ratio of 3) were calculated by FIA at a flow rate of 2 \(\mu\)L/min, using an injection volume of 60 nL. The mobile phase composition was kept at 50% water and 50% acetonitrile. Table 4 shows that SIM LODs for the selected pesticides ranged from 3 to 30 pg, i.e., 0.003–0.03 \(\mu\)g/L for \(\mu\)SPE.

**Calibration Curves**

Calibration plots were obtained by FIA, using 5 points from the LOD values to a concentration >2 orders of magnitude. Linear regression equations and mean standard deviation data were calculated on the basis of 5 replicates for each concentration (Table 4). Good linearity was demonstrated along the range of concentrations considered for both methods.

**Instrumental Precision**

The intraday precision was estimated by analyzing 5 times the 1.6 ng/\(\mu\)L standard solution during a working day. The interday precision was evaluated by analyzing 5 times the standard solution over 1 working week. The repeatability for the selected pesticides varied from 10 to 27% for intraday and from 12 to 32% for interday (Table 4).

**Recovery Experiments**

Recoveries were calculated considering the peak areas relative to the analysis of 5 different spiked samples compared with the peak area relative to the analysis of the standard solution. For \(\mu\)SPE experiments, the standard solution was analyzed under the same chromatographic conditions (trap + analytical column) of the spiked samples to avoid any difference in the ionization process. In fact, each analyte was eluted with the same mobile phase composition in both standard and sample injections.

Table 5 shows the results obtained with Carbograph 1 and C18 SPE cartridges. Recoveries for all the compounds were not complete, even under the best analytical condition using distilled spiked water. Better recoveries were obtained with \(\mu\)SPE using Hypercarb or C18 for all the compounds tested in all the water samples (Table 6). The only exception was represented by benomyl using Hypercarb micro-trap. This compound is probably strongly retained by this adsorbent so that acetonitrile is not able to desorb it efficiently, giving rise to a very broad peak, which is also difficult to quantify, as shown in Figure 3.
Methiocarb and methidathion were completely recovered by μSPE from all the water samples, even canal water; recoveries by traditional SPE were 60–70%. For these compounds, the recovery values were poorly affected by the water matrix.

In contrast, well water and, particularly, canal water gave worse recoveries for methomyl and benomyl, both with traditional SPE and μSPE. In fact, canal water contains humic materials which are known to interfere in the preconcentration step; they interact with the analytes and may compete with them for adsorbent active sites, altering their breakthrough volumes, as reported in literature (27–31). These interactions are known to enhance water solubility (32) of less polar compounds such as pesticides (27, 33, 34), polynuclear aromatic hydrocarbons (35), polychlorinated dibenzodioxines and polychlorinated dibenzofurans (36), and polychlorinated biphenyls (37), thus altering their breakthrough volumes and recovery efficiencies from surface water samples.

The results with micro-traps were better or comparable to those obtained with traditional SPE, confirming the suitability of this technique for real sample preconcentration. In fact, a μSPE trap behaves as a short efficient analytical column, focusing the analytes in a sharp band on its top. On the other hand, the SPE cartridge works well when the analytes have $k'$ close to $\infty$ in the adsorption step (and $k' \approx 0$ in the elution step). In forward flush mode, when these ideal conditions are not obtained, the analytes are distributed in a large band that moves through the cartridge, causing sample loss. In μSPE, due to a higher chromatographic efficiency, this phenomenon is minimized because the analytes are focused in a sharp band.

**Conclusions**

In conclusion, the method fulfills the requirement of a miniaturized system for the injection of dilute samples in micro-LC and could be considered a valid alternative to other preconcentration techniques. Compared to traditional SPE, the μSPE device is convenient for efficiency of recovery, time consumption, solvents and adsorbents needed, and sample handling. This fact implies an economic and safety advantage. Furthermore, it is simple and easy to use. Unfortunately, as with traditional SPE, an ideal adsorbent does not exist, but must be chosen according to the analytical problem. In this case, Hypercarb and C18 were tested, but it is possible to use the micro-traps with other adsorbents to widen the field of application.

**Acknowledgments**

We are grateful to Dionex Corp. for providing the LC micro-column and for its constant support of our work.

**References**


**Table 6. Recovery values (%) and relative standard deviation (%) obtained by μSPE trap for selected pesticides**

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>C18 Distilled water</th>
<th>Well water</th>
<th>Canal water</th>
<th>Hypercarb Distilled water</th>
<th>Well water</th>
<th>Canal water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>65 ± 16</td>
<td>40 ± 28</td>
<td>8 ± 30</td>
<td>82 ± 11</td>
<td>92 ± 14</td>
<td>48 ± 42</td>
</tr>
<tr>
<td>Benomyl</td>
<td>89 ± 15</td>
<td>79 ± 19</td>
<td>65 ± 18</td>
<td>98 ± 13</td>
<td>91 ± 12</td>
<td>82 ± 18</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>87 ± 16</td>
<td>94 ± 15</td>
<td>83 ± 12</td>
<td>98 ± 13</td>
<td>97 ± 15</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>Methidathion</td>
<td>92 ± 16</td>
<td>92 ± 14</td>
<td>94 ± 15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3. Chromatographic separation of spiked distilled water passed through Hypercarb micro-trap.**