Headspace solid phase microextraction in the determination of pesticides in water samples from the Okavango Delta with gas chromatography-electron capture detection and time-of-flight mass spectrometry

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A B S T R A C T

Headspace solid phase microextraction (HS-SPME) was optimized for the analysis of pesticides with gas chromatography electron capture detection (GC-ECD) and high-resolution mass spectrometry. Factors influencing the extraction efficiency such as fiber type, extraction mode and temperature, effect of ionic strength, stirring and extraction time were evaluated. The lowest pesticide concentrations that could be detected in spiked aliquots after HS-SPME-GC-ECD ranged from 0.0005 to 0.0022 μg L⁻¹. Consequently hexachlorobenzene, trans-chlordane 4,4'-DDD and 4,4'-DDE were detected in water samples after HS-SPME at concentrations ranging from 2.4 to 61.4 μg L⁻¹ that are much higher than the 0.1 μg L⁻¹ maximum limit of individual organochlorine pesticides in drinking water set by the European Community Directive. The same samples were cleaned with ISOLUTE C₈ SPE sorbent with an optimal acetone/1-butanol (1:1 v/v) mixture for the elution of analytes. No pesticides were detected after SPE clean-up and pre-concentration. Precision for both methods was satisfactory with relative standard deviations less than 20%. This work demonstrated the superiority of HS-SPME as a sample clean-up and pre-concentration technique for pesticides in water samples as well as the need to identify and control point sources of pesticides.

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1. Introduction

Water, a key constituent of ecosystems, is a recipient of a variety of xenobiotics such as pesticides and industrial chemicals by way of direct discharges from point sources or contaminated storm water run-off [1]. Organochlorine pesticides (OCs), in particular, have a potential to give rise to serious ecological effects in freshwater environments due to their resistance to biological, chemical and photo-degradation [2]. Not only do OCs have lethal toxic effects on aquatic organisms such as fish, but they bioaccumulate and biomagnify up in the food chain and exert carcinogenic and reproductive consequences in animals and human beings [3]. As a result, regulatory bodies such as the European Community have set the maximum concentrations of individual OCs in drinking water at 0.1 μg L⁻¹ and the total amount of pesticides at 0.5 μg L⁻¹ [4]. Maximum individual concentrations for aldicarb, diazinon and heptachlor epoxide have even been set lower at 0.03 μg L⁻¹ [5]. There is therefore, a need for highly sensitive analytical methods involving sample preparation techniques with high pre-concentration capacities for monitoring environmental pollutants especially in water employed for human consumption to keep these levels in check.

Sample preparation is often considered to be a fundamental step in the analytical procedure because it not only helps to achieve the low detection limits set by regulatory authorities by cleaning up the sample matrix but also pre-concentrates analytes of interest from a dilute sample matrix for positive identification [6]. Traditional sample preparation methods for water samples such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) were laborious and consumed considerable amounts of organic solvents [7] however, modern microextraction techniques such as solid phase microextraction (SPME), stir-bar sorptive extraction (SBSE), liquid-phase microextraction (LPME) require minimal handling and consumption of organic solvents as well as offer high selectivity and enrichment factors [8].

SPME, introduced by Pawliszyn in 1989, is a well established solvent-free, easy to use, rapid and portable sample preparation method that is compatible with several analytical instruments such as GC and HPLC [10,11]. It is based on the partitioning of analytes between the sample matrix and the polymeric fiber coating and their subsequent desorption on the injection port of a chromatograph [12]. The major advantage of SPME is that it incorporates sample extraction, clean-up and pre-concentration of a wide variety of compounds from both solid-liquid matrices into a single procedure [13,14]. Since its development, SPME has been applied to the analysis of environmental, food, biological and pharmaceutical samples [15,16].

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The study aims at employing the high enrichment capacity of SPME with particular care in preserving a cold room at 4°C prior to analysis.

2.4. Chromatographic conditions

2.5. GC-EC

2.5.1. GC-EC

2.5.2. GC-ToF-MS

2.6. SPME optimization

Before use, the fibers were conditioned for 2 h in the injection port of the GC-EC according to the manufacturer's instructions while maintaining the GC oven and detector at 200 and 300 °C, respectively. A blank injection was performed to confirm removal of impurities from the GC system. Other blank experiments also carried out were those of fiber blanks, a blank of the fiber inserted into an empty vial, a vial containing 0.5 g NaCl and a vial containing 2 ml of ultra pure water. For all the optimization experiments, a 2 ml aliquot of ultrapure water (adjusted to pH 6.5) was placed into a 4 ml vial and spiked with 20 μl of 10 mg l⁻¹ pesticides standards mixture. Initially, the extractions were carried out at 60°C and the extraction time set to 30 min. Thermal desorption on the GC injection port was carried out at 250°C for 5 min.

The extraction efficiencies of the available fibers were evaluated by direct immersion of the fibers into spiked water aliquots. 65 μm PDMS
DYB fiber gave the highest peak areas and hence it was chosen for the subsequent experiments. Both direct immersion (DI) and headspace (HS) extraction modes were evaluated employing 85 µm PEMS/DVB fiber and the peak areas compared. The effect of temperature on the extraction efficiency was investigated at temperatures 40, 60 and 80 °C. Highest extraction efficiencies were obtained at 80 °C. The effect of ionic strength on the extraction efficiency was investigated by adding 0, 10, 20, 30, 40 and 50% of NaCl (w/v) to the spiked water. The vials were covered and swirled for 1 min to dissolve the salt before extraction. Addition of 10% NaCl gave the highest extraction efficiencies. The effect of agitation on the extraction efficiency was studied at a maximum speed of 300 rpm. A precaution was taken to modify the stirring bar by covering it with glass to prevent absorption of pesticides onto the stirrer coating [22]. No positive effect was observed hence no stirring was employed for subsequent experiments. The extraction profiles of pesticides at 80 °C were reconstructed for different times (15, 30, 45 and 60 min). The optimal time was 30 min.

2.7. SPE optimization

The pH of a 30 ml aliquot of ultra high purity water was adjusted to 6.5. The aliquot was spiked with 100 µl of the 1-50 mg L⁻¹ standard mixture. The ISOLUTE C₁₈ sorbent was conditioned with 2 ml methanol (MeOH) and equilibrated with 2 ml ultra high purity water. The spiked 30 ml aliquot was eluted at a rate of 2 ml/min and the SPE sorbent was dried under vacuum for 30 min. Six elution solvents were investigated namely CH₃OH (100%), CH₃CN (1:1 v/v), CH₃Cl/MeOH (5:1), CH₃Cl/MeOH (1:1 v/v), acetone/hexane (1:1 v/v) and CH₃CN/hexane (1:1 v/v). The eluate was evaporated to complete dryness under a stream of N₂ gas. The analytes were reconstituted in 100 µl acetone/hexane (1:1 v/v). 1 µl was then analysed by GC-ECD and peak areas of each pesticide were compared to those in an equal volume of standard mixture which had been similarly dried and reconstituted. The elution solvent system that gave the highest recoveries for most pesticides was acetone/hexane (1:1 v/v) and hence was chosen for the analysis of water samples.

2.8. Analytical parameters

The linearity of the SPME method was studied by employing aliquots of ultra pure water spiked at concentrations between (0,0001–0,0010) and (0,0100–0,1000) µg L⁻¹ of the standard mixture. Similarly, the linearity of the SPE method was studied using spiked aliquots at concentrations ranging from (10–500) to (1000–5000) µg L⁻¹.
Calibration concentrations ranged between (0.001–0.010) and (10–5 000) μg L⁻¹ for SPME and SPE, respectively. The SPME and SPE method determination limits (SPME and SPE-LODs) were defined as the lowest pesticides concentrations that could be determined from a spiked aliquot of ultra pure water employing SPME or SPE sample preparation procedures based on the GC-ECD signal-to-noise (S/N) ratio of 3:1 for individual peaks.

2.9. Application of optimised HS-SPE and SPE to water samples

The optimised HS-SPE and SPE conditions were applied to the sixty-one water samples (pH adjusted to 6.5) and analysed by GC-ECD while GC-ToF-MS was used for confirmation of analytes.

3. Results and discussion

3.1. SPME optimisation

Results displayed in Fig. 2 show that 65 μm PDMS/DVB fiber gave the highest peak areas followed by 30 μm PDMS and 85 μm PA fibers while 7 μm PDMS fibers performed poorly for most pesticides. Some pesticides such as HCB, α-BHC, β-BHC, γ-BHC, 4,4'-DDD, endrin and 4,4'-DDD were extracted onto the 85 μm PA fiber (that is the most suitable for polar compounds) with efficiencies similar to the 65 μm PDMS/DVB fiber. Methylcyclohexane was the only pesticide that was only extracted onto the 7 μm PDMS with the highest efficiency. As expected for semi-volatile and volatile compounds, HS-SPE was more sensitive than the DI mode since HS sampling eliminates competition for adsorption sites on the fiber coating by non-volatile compounds present in the liquid sample [23].

The addition of 10% (v/v) Nafion® introduced a slight improvement in the extraction efficiency of a majority of pesticides except for β-endojson and methoxychlor whereby the addition of 10% salt more than doubled the extraction efficiency of the fiber. Low recoveries for β-endojson and methoxychlor have been reported for HS-SPE by Lambropoulou and colleagues [24]. Increasing the salt content beyond 30% (w/v) showed a decline of the extraction efficiency for all the pesticides.

3.2. SPE optimisation

Dichlorvos was the least recovered pesticide at 52.8% recovery while 2,4'-DDD had the highest recovery of 117.8% using the acetonitrile/n-hexane (1:1 v/v) elution solvent. The low recovery of dichlorvos may be due to its polar character hence weak retention on the non-polar C18 sorbent as compared to the other pesticides. The acetonitrile/n-hexane (1:1 v/v) solvent system gave the highest recoveries hence it was chosen for further clean up of samples.

Table 1. Analytical parameters obtained after SPME and SPE sample preparation techniques and subsequent analysis of pesticides by GC-ECD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SPME</th>
<th>SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Eq (y = x)</td>
<td>10–50000</td>
<td>0.0005–0.1000</td>
</tr>
<tr>
<td>R²</td>
<td>0.9989–0.9994</td>
<td>0.9989–0.9998</td>
</tr>
<tr>
<td>Le (μg L⁻¹)</td>
<td>500–5000</td>
<td>0.0005–0.0000</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>5.3–15.0</td>
<td>5.2–14.0</td>
</tr>
</tbody>
</table>

3.3. Analytical parameters

Analytical parameters were obtained for SPME and SPE sample preparation methods by the analysis of different spiked ultra pure water samples employing pesticides standard mixtures described in Section 2.8. Linear relationships were obtained between peak areas and the analyte concentrations, with high correlation coefficients (r2>0.9998). Table 1 shows that the limits of detection after SPE ranged from 310 to 5100 μg L⁻¹ for aldrin and α-BHC, respectively. In HS-SPE the 65 μm PDMS/DVB fiber was more sensitive to trans-chlordane as it had the lowest SPE-LD of 0.0005 μg L⁻¹. Precision was determined by reproducibility studies expressed by percent relative standard deviation (RSD) of 3 spiked water aliquots and was less than 15% for both methods. High % RSD values were obtained for compounds that were least recovered in each extraction technique (15.0% for dichlorvos after SPE and 14.8% for β-HCH after HS-SPE) and these could have been caused by poor selectivities of the SPE sorbent and SPME fiber to the analytes in the presence of matrix components González and co-workers [25] recommended employing a matrix-matched calibration rather than the solvent-based calibration so as to reduce variability of pesticides caused by matrix effects. Paskow [27] reported that injecting samples containing traces of water into the GC-ECD could lead to variations in the responses of the analytes. Traces of moisture could have been from the headspace of the water samples during SPE or inadequate drying of the SPE eluates. The variability of the water samples in terms of sampling locations, where sediment composition and vegetation type differ drastically, may have also impacted on the high % RSD.

3.4. Water analysis

3.4.1. Application of optimised SPME method to the water samples

The optimised SPME method was employed to determine pesticides in water samples and four pesticides namely HCH, trans-chlordane, 4,4'-DDE and 4,4'-DDD were detected by GC-ECD at concentrations ranging between 2.4 and 61.4 μg L⁻¹ as shown in Table 2.

Fig. 2. Comparison of extraction efficiencies of five SPME fibers: A = 7 μm PDMS, B = 30 μm PDMS, C = 100 μm PDMS, D = 65 μm PDMS/DVB and E = 85 μm PA. Pesticides are as follows: 1 = HCB, 2 = α-BHC, 3 = β-BHC, 4 = γ-BHC, 5 = hexachlorobenzene, 6 = dieldrin, 7 = trans-chlordane, 8 = 4,4'-DDD, 9 = endrin, 10 = 2,4-DDE, 11 = endrin, 12 = 4,4'-DDD, 13 = β-endojson, 14 = 4,4'-DDT, 15 = methylcyclohexane.
Phthalates (employed in the plastic industry) were also identified in the water samples but could not be quantified due to lack of pure standards. A chromatograph of a water sample after HS-SPME followed by GC-ECO is shown in Fig. 3.

3.4.2 Application of optimized SPE method to the water samples

The optimized SPE conditions were applied to the clean-up of water samples. The general profile of water samples collected along the main channel of the delta – also known as the Panhandle (Mokhonto, Shaktaw, Samochema, Seppa and Guma Lagoon) – differed from that of samples collected downstream (Main, Toteng and Lake Ngami) next to lodges and villages. Compounds identified upstream were absent downstream probably due to degradation or change in pH from near-neutral upstream to alkaline downstream. Table 3 shows compounds detected in samples from the Panhandle and those from downstream after SPE clean-up.

Dodecamethylene glycol oxide is employed in a variety of industrial products including household and car care products as well as chemical formulations [29], DBEHP is one of the main phthalates used as a plasticiser in the production of PVC and has been classified by the Environmental Protection Agency (EPA) as a possible human carcinogen substance [28].

Water samples collected downstream next to lodges and villages showed the presence of hydrocarbons such as dodecane (C₁₂H₂₅), hoxadecane (C₁₃H₂₅), octadecane (C₁₈H₃₇), eicosane (C₂₀H₄₁), and 1,3,5,7-tetramethyl-1,3,5,7-tetradecadiene. Hydrocarbons are naturally found in unpoluted environments as a result of biotransformation of plant materials but may also occur due to contamination from petroleum spills or combustion processes [29]. Low molecular weight hydrocarbons (n-C₁₂–n-C₁₈) are indicative of degradation of plant matter while high molecular hydrocarbons (n-C₂₀–n-C₄₀) suggest possible petroleum contamination [30]. Even though hydrocarbon standards were not available for quantification, the presence of eicosane – a high molecular weight hydrocarbon – in water samples collected next to lodges and villages shows possible petroleum contamination of the delta’s water due to point source pollution.

4. Conclusions

Festicides were determined in water employing optimized HS-SPME with GC-ECO and confirmed by GC-MS. Satisfactory precision was obtained for both HS-SPME and SPE methods. However, HS-SPME exhibited a higher degree of selectivity and sensitivity to load phase with determination limits 3-fold lower than those for SPE. While no pesticides were detected after clean-up by SPE, HCB, trans-chlordane, 4,4′-DDD and 4,4′-DDT were detected with the HS-SPME method. Hence HS-SPME is recommended for environmental monitoring due to its high selectivity and high pre-concentration capacity. The authors recommend that analyses of phthalates and pesticides should be included in regular monitoring programs of the Okavango Delta ecosystem. Sources of contamination need to be identified and controlled to prevent further contamination of the Okavango Delta as well as reduce the present levels of pesticides in the water that are much higher than the recommended EU levels for drinking water.

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