Simultaneous Preconcentration of Oxyfluorfen and Chlorpyrifos in Environmental Water Samples Using Spent Coffee Grounds as SPE Sorbents

Ismail I. Fasfous*, Suha E. Rehan, Jamal N. Dawoud

Department of Chemistry, Faculty of Science, Hashemite University, Zarka, Jordan
P.O. Box 150459, Zarka 13115, Jordan.

Received on Feb. 22, 2012 Accepted on May 24, 2012

Abstract

A new procedure for the simultaneous determination of Oxyfluorfen and Chlorpyrifos pesticides in environmental aqueous samples was developed. It was based on solid-phase extraction (SPE) using spent coffee grounds (a residue from soluble coffee industry and/or coffee shops) as sorbents and high-performance liquid chromatography-ultraviolet detector for the enrichment and determination of the two pesticides at trace level. Some parameters that influence the enrichment efficiency of spent coffee grounds towards those pesticides, such as eluent type and its volume, pH of the water sample, volume of the water sample, mass of the sorbent, and column regeneration were investigated and optimized in detail. Under the optimal SPE conditions, the linear range, detection limit, and precision (R.S.D. n=3) were 0.77-50 ngmL⁻¹, 0.23 ngmL⁻¹, 4.5-0.1% for Oxyfluorfen; 3.21-100 ngmL⁻¹, 0.96 ngmL⁻¹, 1.7-0.3% for Chlorpyrifos, respectively. The proposed method was successfully applied to the analysis of tap water and stream water samples and satisfactory spiked recoveries were obtained in the range of 100.3–106.2 %. The above results indicated that the developed method could be used for the routine analysis in environmental water samples.

Keywords: Pesticides; Spent coffee grounds; Solid phase extraction; Enrichment; Preconcentration; HPLC.

Introduction

Pesticides are used in agriculture, public health, construction, households and are widely distributed in the environment. As a result of the massive annual world consumption (5.2 billion pounds in both 2006 and 2007)¹¹, pesticides have attracted increasing attention all over the world due to their toxicity, persistence, and/or potential adverse effects on the ecosystem even if present at very low concentration levels²³. Many studies have reported the presence of pesticides in air⁴, food⁵,
European Community Directive fixes allowable levels of pesticides in drinking water to 0.1 ng mL$^{-1}$ for individual pesticides and some of their degradation products, and 0.50 ng mL$^{-1}$ for the sum of all pesticides. Therefore, it is of essential importance to set up reliable and efficient methods to determine the residues of pesticides in various water environments at trace levels in terms of commonly used instruments such as high performance liquid chromatography (HPLC) in combination with ultraviolet (UV) or diode array detector (DAD). However, these instruments have insufficient instrumental sensitivity and selectivity for direct determination of pesticides at trace or ultra trace concentration levels in environmental samples. Hence, preconcentration and separation procedure is often necessary to improve the sensitivity and selectivity of determination of the employed technique. Solid phase extraction (SPE) is the most widely used sample pretreatment and enrichment technique prior to chromatographic analysis because of its advantages to high enrichment factor, high recovery, extreme simplicity, rapid, low expense, low organic solvent consumption and the ability to combine with different detection techniques. Activated carbon has been the most popular and widely used as a versatile sorbent in SPE for removal of various types of pollutants such as dyes, metal ions, pesticides, phenols, and many other chemicals. Nevertheless, in recent years and due to the high cost, non-renewable source, and lack of reversible sorption (e.g., regeneration) of commercially available activated carbon, researchers have made great efforts towards developing low-cost alternative adsorbents. Coffee is one of the most popular beverages in the world in terms of consumption. World coffee production was 6.7 million metric tons annually in 1998-2000, and rose to 7 million metric tons annually in 2010. However, significant amount of solid wastes generated from coffee industry such as coffee husks, defected coffee beans (low quality coffee) and spent coffee grounds (from soluble coffee production), have been explored as adsorbents for water treatment. Tokimoto et al. have demonstrated the potential of coffee grounds as adsorbent to remove lead ions from drinking water. Kaikake et al. have studied the feasibility of degreased coffee beans for adsorption of Cu(II), Zn(II), Pb(II), Fe(II) and Cd(II). The results showed that all metals were adsorbed at low pH values 3-5. The desorption recovery of Cd(II) was over 90% using nitric acid or hydrochloric acid.

Oxyfluorfen pesticide (a nitrophenyl ether herbicide) is widely used in agriculture to control broadleaf weeds (e.g., onion, garlic, grapes, etc.) and grass, while Chlorpyrifos pesticide (an organophosphate insecticide) is commonly used for urban and domestic pest control and in agriculture, and sanitation industries worldwide. To our knowledge, up to now, no report about solid-phase extraction using spent coffee grounds as sorbents for the enrichment and determination of Oxyfluorfen and
Chlorpyrifos has been reported in the literature. The main objective of this study is to exploit the analytical potential of spent coffee grounds (could be obtained from coffee shops opened and/or soluble coffee production) as solid-phase extraction sorbent for the enrichment of Oxyfluorfen and Chlorpyrifos at trace level. Related important factors influencing the extraction efficiency, such as eluent type and its volume, mass of spent coffee grounds, pH of the water sample, volume of the water sample, and column regeneration were investigated and optimized. At the same time, the proposed SPE procedure was used for the simultaneous determination of Oxyfluorfen and Chlorpyrifos in tap water and stream water samples prior to their determination by HPLC-UV.

Experimental

Chemicals and Reagents

The compounds studied, Oxyfluorfen (99% purity) and Chlorpyrifos (98% purity), were kindly supplied by Vegetarian Agricultural Production Company/VAPCO (Zarqa, Jordan). The chemical structures and physical and chemical properties of the two pesticides are presented in Table 1. HPLC-grade acetonitrile and water were purchased from Tedia Company Inc. (Fairfield, USA). Nitric acid (Tedia, USA) and sodium hydroxide (GCC, UK) were used to adjust the pH. HPLC water was used throughout the work. All other chemicals used were of analytical reagent grade unless stated otherwise. Commercial HyperSep C18 SPE columns (3mL, 200mg) were purchased from Thermo Scientific Cooperation.

Table 1: Physical and chemical properties of the selected pesticides.

<table>
<thead>
<tr>
<th>Pesticide’s common name</th>
<th>Name (CAS) Molecular formula</th>
<th>Vapor pressure (mPa)</th>
<th>Water solubility (mg L⁻¹)</th>
<th>Partition coefficient (Log Pow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyfluorfen</td>
<td>2-chloro-α,α,α-trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether C₁₅H₁₁ClF₃NO₄</td>
<td>0.027 at 25 °C</td>
<td>0.116 at 25 °C</td>
<td>4.47</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate C₉H₁₁Cl₃NO₃PS</td>
<td>2.7 at 25 °C</td>
<td>1.4 at 25 °C</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Preparation of spent coffee grounds as a sorbent.

Arabica ground coffee (as known as Turkish coffee) was purchased from Brazilian Coffee House (International Mill Company), Amman, Jordan. The coffee grounds were washed several times with boiling water to remove color and soluble compounds, and dried in an oven at 70 °C for 24 hours. Oily substances were
removed from the dried coffee grounds using Soxhlet extraction technique using ethanol as the extracting solvent. At the end of the extraction, the spent coffee grounds were air dried overnight in a fume hood, and then dried in an oven at 90 °C for 12 hour. The dried spent coffee grounds was stored in a brown glass bottle and kept in the desiccators until use. The particle size distribution of the spent coffee grounds was determined by Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., Malvern, UK). The average particle size of a sample was quantified by a volume-weighted mean diameter $D^{[4,3]}$. This value is commonly used as characteristics of particle size distribution and equals to the diameter of the sphere that has the same weight as a given particle. Result of this analysis is presented in table 2.

**Characterization of spent coffee grounds**

Methylene blue (MB)-surface area was estimated as follows$^{[22]}$: 25 mL of aqueous solutions containing 10-100 mg L$^{-1}$ methylene blue were separately contacted with 50 mL glass vials with screw caps each containing 50 mg of the adsorbent. The vials were then capped tightly and agitated in an isothermal water bath shaker (GFL 1083, Germany) with reciprocating motion at 24 ºC for 78 hours to make sure the equilibrium was attained. After that, all vials were placed vertically on a flat surface to ensure the separation between solution and spent coffee grounds. A portion of the supernatant layer was taken, and the remaining concentrations of the MB after equilibrium were determined spectrophotometrically at a wavelength of 614 nm using CECIL 2011-visible spectrophotometer. Langmuir sorption isotherm was used to find the monolayer capacity through which MB- surface area was estimated$^{[22]}$. Calibration curve of MB was linear within the range of 2–6 mg L$^{-1}$ and samples of higher concentrations were further diluted to fit into the dynamic range of the calibration curve. The determination of surface oxides was performed as described by Boehm$^{[23]}$. pH value at the point of zero charge, pH$_{PZC}$, of the spent coffee grounds was determined by mass titration procedure as described by Valdés et al.$^{[24]}$. Elemental analysis of the spent coffee grounds was performed using a EURO EA 3000 elemental analyzer (EuroVetor, Italy). Fourier Transform Infrared Spectrometry (FTIR) spectra of the spent coffee grounds were recorded on a Nicolet – Magna-IR 560 Spectrophotometer using KBr pellets. Before each measurement, the instrument was run to collect the KBr spectrum as blank, which was then automatically subtracted from the sample spectrum.
Table 2. Surface area, zero point charge, surface oxides, and elemental analysis of spent coffee grounds.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>MB surface area (m$^2$/g)</th>
<th>pH_{ZPC}</th>
<th>Volume-weighted mean diameter (µm)</th>
<th>Elemental analysis</th>
<th>Bohem titrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent coffee grounds</td>
<td>135.3</td>
<td>3.9</td>
<td>484.3</td>
<td>%C  49.54</td>
<td>a    3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%H  6.61</td>
<td>b    2.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%N  3.18</td>
<td>c    0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%O  40.67</td>
<td>d    0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a    0.30</td>
<td>e    0.41</td>
</tr>
</tbody>
</table>

(a) Total basic group (mmol g$^{-1}$), (b) total acidic group mmol g$^{-1}$, (c) phenolic groups (mmol g$^{-1}$), (d) lactonic groups (mmol g$^{-1}$), (e) carboxylic groups (mmol g$^{-1}$).

Apparatus

All pH-measurements of water samples were measured using a a (InoLab pH Level 1) pH meter equipped with a combination pH glass electrode (WTW, Germany). Electrical conductivity was determined by a Milwaukee Smart SM302 Portable conductivity meter (Romania). The determination of the two pesticides were carried out using a high performance liquid chromatography instrument (EliteLachrom, Hitachi-Japan) equipped with an UV detector (Model L-2400), a pump (Model L-2130), and an autosampler (Model L-2200). The separations were performed on a Hypersil ODS C$_{18}$ (4.6 x 150 mm, 5 µm) from Thermo Electron Corporation. The mobile phase was a mixture of acetonitrile and water (80:20, $V_{CH,CN} : V_{H,2O}$), and the flow rate of the mobile phase was set at 1.0 mL min$^{-1}$. Before using, the mobile phase was passed through a 0.45 µm membrane filter (ALBET® cellulose nitrate) and degassed in an ultrasonic bath. The measurements were carried out at 25 ± 2 ºC. Chromatographic data were acquired and processed by UV detection L-2400 at 220 nm, using the peak area mode for quantification and determination. The injection volume was twenty microliters.

Stock standard solutions of each individual compound, at concentration of 100 µg mL$^{-1}$, were prepared daily by weighing 10.0 mg of the compound powder and dissolving it in 100 mL acetonitrile and were stored at 4 ºC in the dark. Bicompound working standard solutions (with concentrations between 10 to 15000 ng mL$^{-1}$ for each compound) were prepared daily by corresponding dilution of the stock standard solutions with acetonitrile. Quantification of the pesticides was performed by external calibration, using eight-point calibration curves. Measurements were performed in three replicates and the average of these replicates were reported (n=3).

Instrumentation

In general, the chromatographic parameters (column, mobile phase, mobile flow rate, temperature) were crucial to satisfy the requirements of successful separation and detection of the analytes. The optimization of the spectrometric detection was achieved by injection of the standard solutions containing the two pesticides simultaneously in the range of 10-15000 ngmL$^{-1}$ into the HPLC-UV. Instrument figures
of merit such as limits of detection, limits of quantification, linear ranges, precisions, and regression coefficients ($R^2$) for the developed HPLC-UV method have been calculated and presented in Table 3. Instrumental limits of detection (ILD) and Instrumental limits of quantification (ILQ) were calculated based on signal-to-noise ratio (S/N) 3 and 10, respectively. To find the linear range for each pesticide, the average peak area for each pesticide was plotted against its added concentration and limit of linearity was estimated based on the $R^2$ value of the line. Each pesticide showed linearity in the studied working range, with $R^2$ greater than 0.9993. In figure 1, the typical HPLC chromatogram of an acetonitrile (blank, dotted line) is overlaid with a chromatogram (solid line) of 5 µg.mL$^{-1}$ of the two pesticides standard.

![Figure 1](image-url)

**Figure 1**: Standard HPLC overlaid chromatograms of 5 µg mL$^{-1}$ of the two pesticides. (1), Oxyfluorfen; (2),Chlorpyrifos. The dotted chromatogram represents blank (acetonitrile); the solid chromatogram represents 5 µg mL$^{-1}$ of the two pesticides standard.

**Table 3.** Figures of merit of the instrumental method.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Limit of detection (ng.mL$^{-1}$)</th>
<th>Limit of quantification (ng.mL$^{-1}$)</th>
<th>Linearity range (ng.mL$^{-1}$)</th>
<th>Regression coefficient ($R^2$)</th>
<th>Precision, %RSD range (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyfluorfen</td>
<td>21.5</td>
<td>40.2</td>
<td>40.2-15000</td>
<td>0.9993</td>
<td>2.01-0.14</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>29.1</td>
<td>57.8</td>
<td>57.8-15000</td>
<td>0.9999</td>
<td>2.67-0.15</td>
</tr>
</tbody>
</table>

RSD, relative standard deviation; limits of detection and quantification were based on $\frac{S}{N} = 3$ and 10, respectively. 1500 ng mL$^{-1}$ is the highest pesticide concentration used in determining the linearity range.
Preparation of the column

Solid phase extraction was performed using a visiprep-12-port vacuum manifold from Supelco, Germany. Spent coffee grounds packed cartridge was prepared by placing a specific mass of the spent coffee grounds in an empty 6 mL polypropylene SPE-tube “filtration tube”, Supelco. Polyethylene frits (20 µm porosity) were used to hold the adsorbent packing in the place. In order to reduce the interferences of organic contaminants, the entire SPE assembly needed to be washed with sufficient acetonitrile and water. The flow rate was maintained under gravity (around 3 mL per minute). The packed cartridge was then preconditioned by washing with 5 mL of acetonitrile and then with 10 mL of water ahead of the preconcentration procedure. The conditioning solutions were forced through the adsorbent to remove adsorbed impurities and activate the surface of the adsorbent.

SPE procedure

Stock solution containing the two pesticides at 100 µg.mL⁻¹ was prepared in acetonitrile. The standard solutions (2.5-100 ngmL⁻¹) were prepared by diluting desired volume of stock solution in HPLC water. The desired mass of spent coffee grounds was loaded into the column and pre-conditioned as described above. An appropriate volume of aqueous sample solution containing Oxyfluorfen and Chlorpyrifos was prepared. After that the solution was adjusted to the desired pH value and passed through the column gravitationally. Then the cartridge was washed with 10 mL of 5% of acetonitrile aqueous solution to remove co-adsorbed matrix materials from the cartridge. Subsequently, Oxyfluorfen and Chlorpyrifos retained on the cartridge were eluted with desired volume of eluting solvent. The eluent was analyzed for the determination of pesticides concentrations by HPLC-UV with an injection of 20 µL. This procedure was repeated several times when changing one of the parameters at a time while keeping all other parameters constant in order to reach to the optimal conditions.

Analytical performance of the proposed method

Under the optimal SPE conditions, three hundred milliliters of HPLC water samples were spiked with various concentrations of the two pesticides: 2.5, 10, 25, 50, and 100 ng mL⁻¹ and then enriched using spent coffee grounds. SPE was performed in three replicates and the three extracts were performed as separate samples. The method limits of detection (MLD) and method limits of quantification (MLQ) were applied for preconcentration and analysis method developed for the analysis of the target pesticides within a matrix. MLD and MLQ were calculated as based on \( \frac{3 \times s_{\text{blank}}}{m} \), \( \frac{10 \times s_{\text{blank}}}{m} \), respectively. Where \( m \) is the slope of the calibration curve.
of the developed method and $s_{\text{blank}}$ is the standard deviation of the repetitive measurements on a method blank.$^{[25]}$

**Water samples**

Two environmental water samples were used for evaluation of the proposed SPE method including tap water and stream water using grab sampling with brown glass bottles (borosilicates) and were brought to our laboratory without any delay. Stream water samples were collected from Zarqa stream water (Zarqa, Jordan) in November 2010. Tap water samples were taken after flowing for 10 min. from various water taps in our laboratory in different days, and then pooled and used to generate a composite tap water sample. Before use, all the environmental water samples were filtered through 0.45 $\mu$m micropore membranes (ALBET® cellulose nitrate membrane filter) to remove particulate matter and stored in brown glass bottles at 4 °C. The pH and conductivity of the samples were measured: pH=7.4 and conductivity = 1.4 mS for stream water samples and pH=7.8 and conductivity = 0.5 mS for Tap water samples. The optimal SPE procedure was applied as described above and the eluent was analyzed by a HPLC-UV system. SPE experiments were performed in three replicates ($n = 3$) and the three extracts were analyzed as separate samples.

**Results and discussion**

*Physical and chemical properties of spent coffee grounds*

The chemical and physical properties of spent coffee grounds including functional groups, elemental analysis and surface area were determined using standard techniques and presented in table 2. With methylene blue surface area of 135.3 m$^2$ g$^{-1}$, the spent coffee ground is a potential sorbent for the selected pesticides (see Table 1). Baek et al.$^{[26]}$ have reported that the surface area of degreased coffee beans is 173.2 m$^2$.g$^{-1}$ using N$_2$ gas adsorption technique. They have found that treating raw coffee beans with sodium hydroxide solution increases the surface area, porosity and adsorption capacity of the raw coffee beans. Our result is presumably due to probable change in the microporous structure of the spent coffee grounds. With application of successive Soxhlet extraction on spent coffee grounds, the oily substances of raw coffee grounds reduce. Oily substances act as a film on the surface of coffee materials as reduce its porosity. Therefore, treatment with Soxhlet extraction (ethanol as extracting solvent) probably enhances the surface area of raw coffee grounds, making it more porous. Bohem’s titration revealed that the spent coffee grounds have both acidic and basic properties. The acidic functional groups are carboxylic, lactonic, and phenolic. The basic functional groups include oxygen-containing moieties such as ketonic, pyronic, chromenic$^{[23]}$. The spent coffee grounds has acidic surface properties and has a relatively high density of surface functional
groups (14.69 group/ nm$^2$) compared to reported value for activated carbon (0.54 group/ nm$^2$ [27]). In fact, carboxylic group attracted to carbon surface has a pK$\text{a}$ value within 4-5. Therefore, this functional group would accrue different charges with respect to the studied solution pH while phenolic groups (2.07 mmol g$^{-1}$) will be deprotonated after pH 10. The distribution of the functional groups and chemical composition in the surface of the spent coffee grounds play a major role in determining its capacity, affinity and selectivity towards specific analytes (e.g., pesticides). However, the interaction of the pesticides with all functional groups is possible via many sorption mechanisms including; electrostatic interaction, hydrophilic-hydrophilic, and hydrophobic-hydrophobic modes.

The point of zero charge (pHpzc) of spent coffee grounds is 3.9 and therefore pH values should be carefully controlled in order to have a predominant positively (pH<$\text{pH}_{\text{pzc}}$), neutral (pH=$\text{pH}_{\text{pzc}}$) or negatively charged surface (pH>$\text{pH}_{\text{pzc}}$) of the sorbent. The pH of the spent coffee grounds suspension is quite similar to the value of pH$\text{pzc}$ (~3.9), which is in agreement of with high oxygen contents. Escudero et al. [28] have reported the value of pH$\text{pzc}$ = 3.9 for exhausted coffee waste obtained from soluble coffee production industry.

The FTIR spectrum of spent coffee grounds showed a decrease in the intensity of transmittance peaks (%T) in the range of 400 cm$^{-1}$ to 4000 cm$^{-1}$, compared with control spectrum (as-received ground coffee). The control spectrum showed as a peak at 1742.5 cm$^{-1}$ due to the resulting carboxyl linkage derived from the xanthine derivatives such as caffeine. Since this transmittance peak was not observed in the spectrum of spent coffee grounds, it is likely that coffee ingredients, such as caffeine, were removed by Soxhlet extraction procedure. According to Kaikake et. al. [17], similar results have been reported.

**Optimization of SPE procedure**

Optimization of SPE procedure is an important process to get appropriate enrichment efficiency and full recovery. It usually involves pH of the sample, solution volume, mass of spent coffee grounds, eluting type and its volume and the concentration of pesticides in solution. Spent coffee grounds as a SPE sorbent for concentrating organic pollutants had not been reported and no related researches on the trapping pesticides were found. Hence it was reasonable to carry out several series of experiments for obtaining optimal enrichment conditions. All experiments were performed in three replicates ($n=3$) and RSD was less than 4.5%.

**Effect of water sample pH**

The pH plays an important role in determining the extent of pesticides retained on spent coffee grounds since the variation of the pH affects both the speciation and
stability of the pesticides as well as the surface properties (e.g. protonation of the functional group responsible for sorption) of the spent coffee grounds. A series of experiments were executed to investigate the influence of sample pH over the range of pH 3.0-11.0 on the recoveries of the two pesticides dissolved in aqueous solutions using 200 mg spent coffee grounds as a SPE adsorbent, and a loading volume was 100 mL of HPLC water spiked with 100 ng.mL⁻¹ of each pesticide. The pH of solutions was adjusted to a certain pH value by a dropwise addition of 0.01M HNO₃ or 0.01 M NaOH as required.

![Figure 2](image)

**Figure 2** Effect of pH of the water sample on recoveries (%) of the two pesticides. 100 mL HPLC water sample spiked with 100 ngmL⁻¹ of the two pesticides; mass of spent coffee, 200 mg; washing solvent, 10 mL of 5% acetonitrile aqueous solution; eluent volume, 10 mL acetonitrile. □, Oxyfluorfen; ○, Chlorpyrifos.

Results and details of the experimental conditions are shown in figure 2. It was noted that the highest recoveries were observed at pH 7.0 for Oxyfluorfen and Chlorpyrifos pesticides. Therefore, pH 7.0 was considered the optimal pH in this study. All subsequent studies were carried out at pH 7.0 for well extraction based of the characteristics of the two pesticides.

**Type of eluting solvent**

As pesticides on the SPE cartridge was concerned, different degrees of performance would be achieved due to the different elution power of the various
solvents. Herein two organic solvents namely, acetonitrile and methanol were compared for the optimal selection. In the experiment, HPLC-grade acetonitrile and methanol was used directly as obtained. The analysis for the concentration of the target analytes were measured in the each eluting solvent to determine the % desorption of pesticides from spent coffee grounds. Figure 3 shows the effect of acetonitrile and methanol as eluting solvents, on the elution of pesticides from spent coffee grounds. The graph shows that the order for overall elution performance was for acetonitrile > methanol. This is probably due to the hydrophobicity of SCG as the relatively non-polar sorbents, the desorption of pesticides (as non-polar solutes) using acetonitrile was more efficient than methanol due to the lower polarity of the acetonitrile compared with methanol. This may enable the acetonitrile to be associated with the SCG much more than methanol. As a result, acetonitrile was selected as the eluent in the following experiments.

**Figure 3** Effect of the eluent type on % recovery of the two pesticides. 100 mL HPLC water sample spiked with 100 ng mL⁻¹ of the two pesticides; pH of the sample solution, 7; mass of spent coffee, 200 mg; washing solvent, 10 mL of 5% acetonitrile aqueous solution.

**Eluting volume:**

It is important to be sure that all the adsorbed species in the cartridge were removed, but at the same time the recovery of the analytes must not be affected. Thus, a series of experiments was designed to get the proper volume of acetonitrile as eluting solvent without loss of extraction efficiency. Results of studying the effect of
eluting solvent volume on % recovery of the pesticides with appropriate experimental conditions are exhibited on figure 4. It was found that volume of the eluent would directly affect the extracting efficiency of Oxyfluorfen and Chlorpyrifos. As shown in figure 4, 10 mL of acetonitrile was sufficient to elute all Oxyfluorfen, while 8 mL of acetonitrile gave the highest recovery for Chlorpyrifos. However, we decided to use an elution volume of 10 mL to have highest recovery for both pesticides.

Figure 4 Effect of eluent volume (acetonitrile) on recoveries (%) of the two pesticides. 100 mL HPLC water sample spiked with 100 ng/mL of the two pesticides; pH of the sample solution, 7; mass of spent coffee, 200 mg; washing solvent, 10 mL of 5% acetonitrile aqueous solution. □, Oxyfluorfen; ○, Chlorpyrifos.

Effect of the mass of adsorbent:

In our paper the adsorbent was the spent coffee grounds so it is important to show the effect of changing the mass of coffee grounds with the % recovery. The effect of varying mass of the spent coffee grounds on the recovery of the two pesticides was studied using the mass of 200, 300 and 400 mg as shown in figure 5. Oxyfluorfen has shown no significant recovery variations with 200, 300 and 400 mg of sorbent. Chlorpyrifos was found to have the highest recovery at 300 mg. However, 300 mg of spent coffee grounds was used for further experiments.
Figure 5 Effect of the mass of spent coffee grounds on recoveries (%) of the two pesticides. 100 mL HPLC water sample spiked with 100 ngmL⁻¹ of the two pesticides; pH of the sample solution, 7; washing solvent, 10 mL of 5% acetonitrile aqueous solution; eluent volume, 10 mL acetonitrile. □, Oxyfluorfen; ○, Chlorpyrifos.

Effect of the sample volume loaded:

In order to investigate the possibility of enrichment of trace quantities of the studied analytes, the effect of sample volume on the recovery was also studied by passing various volumes: 100, 200, 300, 400, and 500 mL. The results and experimental conditions are shown in figure 6. The maximum recovery as well as the enrichment efficiency was reached when passing 400 mL of 25 ngmL⁻¹ of the studied pesticides through spent coffee grounds packed cartridge under gravity. However, no remarkable differences were found among the recoveries (or enrichment efficiencies) of each pesticide over the range of 100-400 mL of sample volume. Taking into consideration enhancing the enrichment factor, reducing analysis time and applicability for routine analysis, 100 mL was adopted as the sample volume for the rest of SPE optimizing procedures.
Figure 6 Effect of sample volume loaded on recoveries (%) of the two pesticides. Appropriate volume of HPLC water sample spiked with 25 ngmL$^{-1}$ of the two pesticides; pH of the sample solution, 7; mass of spent coffee, 300 mg; washing solvent, 10 mL of 5% acetonitrile aqueous solution. □, Oxyfluorfen; ○, Chlorpyrifos.

Column regeneration

This part discusses the reusability of SCG in several experiments. As shown in figure 7, the recoveries of the two pesticides in three successive experiments were high and nearly close to each other. Thus, the same spent coffee grounds can be reused (or generated) in several experiments and keeping reasonable recoveries.

Figure 7 Effect of the column regeneration on the % recoveries of the two pesticides. 50 mL HPLC water sample spiked with 25 ngmL$^{-1}$ of the two pesticides; pH of the sample solution, 7; mass of spent coffee grounds, 300 mg; washing solvent, 10 mL of 5% acetonitrile aqueous solution.
Analytical performance of the developed method

Under the above optimum conditions described in section 3.2, some characteristics of the proposed method were investigated for simultaneous quantitative analysis of Oxyfluorfen and Chlorpyrifos. Method Limits of detection, method limits of quantification, linear ranges, precisions, and regression coefficients ($R^2$) were obtained. A series of experiments with spiked water solutions have been utilized for the related parameters and the results are presented in Table 4. The method limits of detection of two pesticides, were estimated as three-standard deviation of the blank signal for an extraction of 300 mL divided by the calibration curve slope, were 0.23 and 0.96, ng mL$^{-1}$ for Oxyfluorfen and Chlorpyrifos, respectively. The results indicated that the precision was satisfactory at very low level and relative standard deviations (R.S.D.) were 4.5-0.1% and 1.7-0.3%, (n=3) for Oxyfluorfen and Chlorpyrifos, respectively. The sensitivity of the method can be further improved if more than 300 mL sample was used.

Table 4: Figures of merit for the proposed analytical method.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Limit of detection (ng mL$^{-1}$)</th>
<th>Limit of quantification (ng mL$^{-1}$)</th>
<th>Linearity range (ng mL$^{-1}$)</th>
<th>Regression coefficient ($R^2$)</th>
<th>Precision, %RSD range (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyfluorfen</td>
<td>0.23</td>
<td>0.77</td>
<td>0.77-50</td>
<td>0.9728</td>
<td>4.5-0.1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.96</td>
<td>3.21</td>
<td>3.21-100</td>
<td>0.9888</td>
<td>1.7-0.3</td>
</tr>
</tbody>
</table>

RSD, relative standard deviation; limits of detection and quantification were based on $\frac{3 \times s_{blank}}{m}$, $\frac{10 \times s_{blank}}{m}$, respectively.

Application of the proposed method for real water samples

In order to demonstrate the applicability and the reliability of the proposed method, two real environmental water samples including tap water and stream water were used to validate the developed method. The two water samples were spiked with two pesticides at 25 µg L$^{-1}$ for Oxyfluorfen and Chlorpyrifos, respectively. The optimal SPE procedure was applied as described above and the eluent was analyzed by a HPLC-UV system, and the results are given in table 5. The overlaid chromatograms of the blank (unspiked water) and spiked water samples from the proposed SPE method were presented in figure 8. The results indicate that the contents of Oxyfluorfen and Chlorpyrifos in the samples were under the detection limits of the developed method. The recoveries of Oxyfluorfen and Chlorpyrifos obtained from tap water and stream water samples were satisfactory[29], and they were in the range of 100.3-106.2%, and 102.5% for Oxyfluorfen and Chlorpyrifos, respectively. These results indicate that the developed method could be used in environmental water sample analysis. A series of
experiments were executed for comparison of spent coffee grounds with commercial C-18 as preconcentrating sorbent for the two studied pesticides. The obtained results demonstrated that spent coffee grounds showed similar performance to that of C-18 for the two pesticides (data not shown).

Table 5: Percent recoveries of real water samples spiked with 25 ngmL⁻¹ for Oxyfluorfen and Chlorpyrifos determined by the proposed method (n=3).

<table>
<thead>
<tr>
<th>Water samples</th>
<th>Oxyfluorfen</th>
<th>Chlorpyrifos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>106.2±4.4</td>
<td>102.5±2.8</td>
</tr>
<tr>
<td>Stream water</td>
<td>100.3±1.0</td>
<td>102.5±3.6</td>
</tr>
</tbody>
</table>

± represents the standard deviation of three separate experiments.

Figure 8: The overlaid chromatograms from the proposed SPE method. (A), HPLC water; (B), tap water; (C), stream water; 300 mL real water samples spiked with 25 ngmL⁻¹ of the two pesticides; 300 mg spent coffee grounds; washing solvent, 10 mL of 5% acetonitrile aqueous solution; eluting solvent, 10 mL acetonitrile; pH= 7.0. (1), Oxyfluorfen; (2), Chlorpyrifos. The dotted chromatograms represent the unspiked water samples (blanks); the solid chromatograms represent the spiked water samples.
Conclusions

In this paper, a cheap, simple and reliable method was developed for the enrichment and simultaneous determination of two pesticides in environmental water samples based on using spent coffee grounds as solid-phase extraction sorbent prior to HPLC-UV analysis. The limits of detection of the proposed SPE-HPLC-UV method were 0.23, 0.96 and ng mL$^{-1}$ for Oxyfluorfen and Chlorpyrifos, respectively. The results demonstrated that spent coffee grounds exhibited excellent merits as solid-phase extraction sorbent for trapping Oxyfluorfen and Chlorpyrifos, at trace level from environmental water samples. The recoveries of Oxyfluorfen and Chlorpyrifos, obtained from tap water and stream water samples were in the range of 100.3-106.2%, and 102.5% for Oxyfluorfen and Chlorpyrifos, respectively. In a word, it can be concluded that the spent coffee grounds has great potential for the enrichment and determination of more other pesticides in environmental water samples.

Acknowledgments

The authors wish to thank the Deanship of Graduate Studies and Scientific Research / Hashemite University for the financial support and to the administrations of VAPCO (Zarka, Jordan) for providing the pesticides and for the use of their laboratory instruments and equipment. The technical assistance of Mr. Nedal Abu Farha is greatly appreciated.

References