Original Research Article

Molecularly imprinted polymer solid-phase extraction for the analysis of organophosphorus pesticides in fruit samples

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A R T I C L E   I N F O

Article history:
Received 28 October 2012
Received in revised form 2 July 2013
Accepted 23 September 2013

Keywords:
Organophosphorus pesticides (OPPs)
Molecularly imprinted polymers (MIPs)
Food samples
Food analysis
Food composition
HPLC
MIP-SPE
C\textsubscript{18}-SPE
NIP-SPE
Food safety
Food contamination
Agricultural practices
Maximum residue limits in food
Pesticide in food

A B S T R A C T

A new selective material based on molecularly imprinted polymers (MIPs) was prepared and used as solid-phase extraction (SPE) sorbent for sample enrichment of organophosphorus pesticides (OPP) residues prior to high performance liquid chromatography (HPLC). Three OPPs widely used in agriculture (diazinon, quinalphos and chlorpyrifos) were selected as target analytes. Various parameters affecting the extraction efficiency of the imprinted polymers were evaluated to optimize the selective preconcentration of OPPs from water samples. Under the optimized conditions, the developed MIP-SPE method showed excellent linearity in the range of 4–200 \( \mu g \) L\(^{-1}\) with coefficient of determination \((r^2) > 0.997\) and good OPP recoveries of >91% and limits of detection (LODs) ranging from 0.83 \( \mu g \) L\(^{-1}\) to 2.8 \( \mu g \) L\(^{-1}\), which is much lower than the maximum residue limits (MRLs) set by the Codex Alimentarius Commission and Japan Food Chemical Research Foundation. The developed method was successfully applied to the analysis of OPPs in selected fruit samples. MIP-SPE showed superior extraction efficiency towards the OPPs as compared to non-imprinted polymer solid-phase extraction (NIP-SPE) and commercial C\textsubscript{18}-SPE methods.

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1. Introduction

A molecularly imprinted polymer (MIP) is a polymer that is formed in the presence of a molecule (called template) that is extracted afterwards, thus leaving complementary cavities behind (Glad et al., 2000). MIPs are tailor-made materials with high selectivity for target molecules. Many MIPs have been prepared and utilized mainly as affinity chromatography media. The resulting imprinted polymers are stable and robust. In addition, it is notable that the synthesis of MIP is also relatively cheap and easy as compared with other selective materials such as immunosorbent (IS), thus making MIP a clear alternative to the use of natural receptors (Pichon, 2007).

One of the most exciting applications of MIPs is as sorbent for solid-phase extraction (SPE). In SPE, the sample is passed through a cartridge or a packed column filled with a solid sorbent where the analytes are absorbed and then eluted with an organic solvent. This procedure presents several advantages: particularly it is less time consuming than liquid–liquid extraction (LLE) procedure; it decreases the use of toxic solvents and offers the possibility of automation (Andersson, 2000; Pichon and Hugon, 2008). Despite their attractive features, the classical SPE sorbents such as C\textsubscript{18}, ion-exchange and size-exclusion phases are lacking in selectivity towards target analytes. In order to overcome this drawback, the use of MIPs in SPE (MIP-SPE) has been developed (Jiang et al., 2008;
Caro et al., 2006; Han et al., 2005; Fang et al., 2005; Boer et al., 2002). MIP-SPE allows not only the analyte to be pre-concentrated but also the other compounds present in the sample matrix to be removed.

Over the past 60 years, farmers and growers have been using pesticides for food production in order to meet the expectations of consumers, increasing production and quality in food production. As a result, consumers are exposed to pesticides, usually in small quantities in a number of food groups such as vegetable, fruits and juices. Organophosphorus pesticides (OPPs) are one of the most common classes of pesticides involved in poisoning because of the inhibition of acetyl-cholinesterase (Sultaños, 2008). Monitoring and analysis of trace levels of OPPs in food and environmental contamination are therefore essential for human health protection and environmental control. The maximum residue limits (MRLs) of pesticide residues in fruits and vegetables set by the European Unions depend on the type of fruit or vegetables (European Economic Community (EEC), 1976). However, the regulation stated a default limit of 0.01 mg kg\(^{-1}\) for all pesticides combinations in food which has no set MRLs (European Communities, 2005).

OPPs are important compounds to analyze since contamination of drinking water and agricultural products has become a major concern as the number and use of OPPs is steadily increasing. In this work, a new MIP was synthesized based on O,O-diethyl-O-2-quinoxalinyl phosphorothioate (quinalphos) as a template for use in MIP-SPE sample enrichment of selected OPPs, namely quinalphos and two other OPPs with similar structure (diazinon and chlorpyrifos) in fruit samples prior to HPLC-UV analysis. To the best of our knowledge, no report has been published on MIP-SPE of these OPPs.

2. Materials and methods

2.1. Reagent and chemicals

Organophosphorus pesticides (OPPs), diazinon (97.5%), quinalphos (96%) and chlorpyrifos (99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Structures of the analytes are presented in Fig. 1. Hexaconazole (used as internal standard) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Methacrylic acid (MAA, 98%, Fluka Analytical, Steinheim, Germany) and ethylene glycol dimethacrylate (EGDMA, 98%, Sigma–Aldrich, St Louis, MO, USA) were purified prior to use for removing the stabilizers. Azobisisobutyronitrile, AIBN (98%) was purchased from Molekula (Dorset, UK) and recrystallized from methanol. Methanol and acetic acid were obtained from Merck (Darmstadt, Germany) and QReC-Brightchem (Penang, Malaysia), respectively. HPLC grade acetonitrile was obtained from J.T. Baker (Phillipsburg, NJ, USA). Microsyringe (10 μL) was purchased from SGE (Sydney, Australia). Stock solutions of 1000 mg L\(^{-1}\) of each OPP were prepared in acetonitrile. Working solutions were prepared by diluting the stock solutions with acetonitrile. The stock solution and working solutions were stored in the refrigerator at \(-4^\circ\)C when not in use. Double-distilled deionized water of at least 18 MΩ was purified by Simplicity Water Purification System, Millipore (Molsheim, France).

2.2. Instruments

SPE Vacuum Manifold with accessories was purchased from International Sorbent Technology Limited (Hengoed, United Kingdom). SPE-CT\(_\text{18}\) cartridge (3 mL cartridge, 100 mg sorbent), empty SPE cartridge (3 mL cartridge) and PTFE frits (porosity 10 μm) were supplied by Supelco Inc. (Bellefonte, PA, USA). The HPLC system consisted of a JASCO PU-980 HPLC (Tokyo, Japan) pump for mobile phase delivery coupled with a column oven of Shimadzu GC-8A Gas Chromatography (Kyoto, Japan). Samples were injected into the system using a 25 μL loop for sample introduction. Shimadzu SPD-6A UV detector (Kyoto, Japan) was used to detect the analyte peaks and data were interpreted using Power Chrome software TotalChrom Navigator Series 280 (Denistone East, Australia). The column used in this study was a 3 μm Diamond Bond C\(_\text{18}\) 150 mm × 2.1 mm i.d. (ZirChrom Separation Inc., Anoka, MN, USA). Isocratic elution was carried out with acetonitrile-water (60:40, v/v) as the mobile phase at a flow rate of 0.4 mL min\(^{-1}\). Column oven temperature was set at 60 °C. The detection of analytes was carried out with the UV detector at 200 nm (Sanagi et al., 2011).

The functional group for the polymer was analyzed by using a Perkin Elmer 1600 Series Fourier transform infrared spectrometer (FTIR) instrument (Waltham, MA, USA). The elemental analysis of the MIP was determined using a Finnigan CE 125 CHN analyzer (Milan, Italy). The surface and morphology of the MIP was characterized using a JEOL JSM-6701F Field Emission Scanning Electron Microscope (FESEM) (Midland, Canada). Pore size, surface area and pore volume analyses were carried out using a nitrogen surface area analyzer (Mieromericits ASAP 2000, North Huntingdon, PA, USA). Ultraviolet analyses of selected organophosphorus pesticides were recorded using a Shimadzu Ultraviolet–visible (UV–vis) recording spectrophotometer (Tokyo, Japan).

2.3. Preparation of polymers

Quinalphos (0.2983 g, 1 mmol) as template, and methacrylic acid (MAA, 0.3444 g, 4 mmol) as functional monomer was dissolved in 6 mL of acetonitrile (ACN) in a thick wall round bottom flask and then ethylene glycol dimethacrylate (EGDMA, 3.9644 g, 20 mmol) as cross-linker and azobisisobutyronitrile (AIBN, 0.108 g, 0.66 mmol) as initiator were added into the solution. The round bottom flask was placed in ice and deoxygenated with nitrogen for 15 min, then sealed under vacuum. Polymerization reaction was then carried out at 60 °C for 24 h in a thermostat-controlled water bath. The resultant hard bulk polymers were crushed, ground, and sieved through 75 μm sieve. The polymer particles obtained were washed with a mixture of methanol–acetic acid (9:1, v/v) successively in Soxhlet apparatus until template could not be detected by UV spectrophotometry.

![Fig. 1. Chemical structures of the target analytes.](image-url)
The extracted particles were then washed with methanol to remove residual acetic acid. Finally, the collected particles were dried at 55 °C in oven under vacuum for 12 h. For comparison, non-imprinted polymer (NIP) was prepared and treated in exactly the same way except that the template molecule was absent in the polymerization step (Sanagi et al., 2011).

2.4. Molecularly imprinted polymer–solid-phase extraction procedure

A 3 mL cartridge was placed in a 12-port SPE vacuum manifold and was conditioned by passing 10 mL of methanol and followed by 10 mL of deionized water. Spiked water sample (10 mL of 50 μg L⁻¹ of mixed OPPs solution) was slowly passed through the cartridge at a flow rate of 1 mL min⁻¹. The solid phase in the cartridge was not allowed to dry at any time of the analysis.

After loading the sample into the SPE cartridge, 5 mL of washing solvent was applied to remove matrix interferences. The cartridge was dried by passing air for 5 min. The pesticides were eluted (6 mL) from the solid phase with the desired solvent. The eluent was dried under nitrogen gas flow until dryness. Finally, the residue obtained was reconstituted with 50 μL of acetonitrile and injected into HPLC. Analyses of a blank sample (NIP-SPE) and commercial C₁₈-SPE performed using the same procedure was performed for comparison.

2.5. Sample preparation

Fruit samples (150 g) of grapes and green apples were chopped and homogenized using blender with microcutters (National, Matsushita Electric Co. Bhd. Malaysia). A portion of sample (10 g) was placed in Erlenmeyer flask and homogenized with 10 mL of methanol and 10 mL of water by sonication for 15 min. The resulting suspension was filtered through a Buchner funnel and the filter cake was washed with 10 mL of deionized water. The filtrate was used as a real sample for the extraction, through MIP-SPE. The filtrate was also spiked with 0.25 mg L⁻¹ of each diazinon, quinalphos, and chlorpyrifos, and made up to 100 mL with deionized water. The spiked sample (10 mL) was passed under vacuum through an MIP-SPE, NIP-SPE, C₁₈-SPE cartridge (100 mg), using their respective extraction conditions.

3. Results and discussion

3.1. Characterization of the MIP

3.1.1. FTIR analysis

FTIR characterization was performed to determine the functional groups in MIP before and after the washing stage and also in NIP by using the KBr pellet method (Fig. 2): A C=O stretching vibration absorption occurs in the region 1700–1750 cm⁻¹ because the polymer cross-linked polymerization of EGDMA and MAA, and repeated EGDMA as cross-linking unit was in place. The absorbance peaks for all spectra (MIP before and after the washing stage and NIP) are identical except for the intensity. Absorbance at 3600–3400 cm⁻¹ indicates O–H band, where the intensity for MIP before washing is lower than that for MIP after washing, while it is relatively similar to that for NIP. A plausible reason for this phenomenon is that the template molecule (quinalphos) is assembled with monomer (MAA) via hydrogen bonding with hydroxyl group during the preparation of MIP prior to washing. However, after the template removal, a strong and broad stretching vibration absorbance peak of hydroxyl group from monomer is clearly observed due to the absence of any hydrogen bond disruption. Similar results were observed by Brune et al. (1999). The decrease in intensity indicates that the template has been leached out by the washing step.

3.1.2. Elemental analysis

Elemental analysis was carried by CHN analyzer to observe the percentage of carbon (C), hydrogen (H) and nitrogen (N) element in sample A (imprinted polymer before template removal), sample B (imprinted polymer after template removal) and sample C (non-imprinted polymer). The characterization by CHN was carried out with the addition of vanadium pentoxide to each polymer to assist

![Fig. 2. FTIR spectra of MIP (a) before washing, (b) after washing, (c) NIP.](image-url)
in the combustion of the polymers studied. The result showed that the percent of CHN elements for all samples were essentially identical to the theoretical or calculated elements in the samples (Table 1). The presence of nitrogen in sample A can be considered as the successful binding via hydrogen bonding between the polymer and template which contained nitrogen in the molecule structure. Conversely, the decreasing number of nitrogen in sample B was obtained as the template has been removed by the washing step. For comparison, non-imprinted polymer was also analyzed. The small amount of nitrogen in sample B and C in CHN analysis can be explained as the existence of organic cyanide (CN) from AIBN initiator.

### 3.1.3. Field emission scanning electron microscopy

Field emission scanning electron microscopy (FESEM) was used to determine the surface morphology and image of the MIP. Fig. 3 shows SEM micrographs of MIP. The images showed a rough MIP surface with irregular order. A rough and porous surface is expected to enhance the extraction efficiency of MIP as observed by Song et al. (2009).

### 3.1.4. Nitrogen adsorption

Nitrogen adsorption analysis of Brunauer–Emmett–Teller (BET) was used since specific surface area, pore volume, and pore size of polymer strongly influence the efficiency of adsorption. Table 2 shows the results for both MIP and NIP. It is clearly proven that the MIP has larger BET surface area, pore volume and pore size than the NIP due to the existence of imprinting effect in polymer where the MIP has more binding sites distributed throughout the cavity (Bruggemann, 2001). In general, a higher total pore volume of MIP is correlated to a superior sample load capacity. This method is principally useful to determine the size of polymer. Since the pore size of samples was in the range of 2–50 nm, both polymers were defined as mesoporous structure (Cormack and Elorza, 2004).

#### 3.2. Optimization of extraction conditions

The MIP-SPE process was optimized in terms of the washing solvent, volume of loading, the composition and volume of the eluting solvent to achieve good sensitivity and precision of this method.

##### 3.2.1. Effect of washing solvent

In MIP-SPE and NIP-SPE step, the type of the washing solvent plays a vital role. It ensures a selective extraction before elution by removing the matrix component in polymeric matrix and reducing non-specific interaction at binding site. Therefore, the interaction between the analytes and binding sites may be retained maximally (Masquee et al., 2001; He et al., 2007).

The shape and character recognition is usually greatest if the solvent used is the porogen for the polymerization of the MIP. The porogen is also often used as the washing solvent for selective adsorption. For that reason, acetonitrile was used as washing solvent in combination with water.

Experiments were carried out with spiked water (10 mL) containing 0.05 mg L⁻¹ quinalphos loaded onto the cartridges and washed with various acetonitrile–water ratio and eluted with 6 mL 10% of acetic acid in methanol based on previous similar work (Zhu et al., 2007). The concentration recoveries were determined by HPLC. The effect of washing with various percentage of acetonitrile in the acetonitrile–water mixtures (10, 20, 30, 40 and 50%) on the recovery of quinalphos were studied and it was concluded that washing solvent containing 10% and 20% acetonitrile in mixture had no significant effect on the recovery of quinalphos on both MIP and NIP cartridges. However, with 30% of acetonitrile in the washing solutions, the recovery of quinalphos in NIP cartridge was markedly decreased to 43.9%, while the recovery of quinalphos by the MIP cartridges was essentially unchanged (92.3% recovery). This indicates the presence of specific interactions taking place in the binding sites. However, higher portions of acetonitrile in mixture solvent (>40%) led to a large decrease of quinalphos recovery on both the MIP and NIP cartridges due to the disruption of specific interactions between the analytes and binding sites. Therefore, a mixture of acetonitrile–water 30:70% (v/v) was selected as the washing solvent and used in subsequent experiments.

##### 3.2.2. Effect of loading volume

In order to determine the optimum loading volume, the experiments were carried out using different loading volumes ranging from 5 mL to 25 mL. The samples were washed with a mixture of acetonitrile–water 30:70% (v/v) (5 mL) and eluted with 10% of acetic acid in methanol (6 mL). When the sample volumes were 5, 10, 15, 20 mL, the recoveries were 75%, 93%, 98%, 31%, respectively. From the results, the analyte recovery increased from loading volume of 5 mL to 10 mL, beyond which the recovery decreased dramatically. The recovery of analyte

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>C (%)</th>
<th>H (%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Theoretical</td>
<td>55.78</td>
<td>6.35</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>54.53</td>
<td>6.30</td>
</tr>
<tr>
<td>B</td>
<td>Theoretical</td>
<td>61.27</td>
<td>7.43</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>61.11</td>
<td>7.10</td>
</tr>
<tr>
<td>C</td>
<td>Theoretical</td>
<td>61.27</td>
<td>7.43</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>61.06</td>
<td>7.38</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>MIP</th>
<th>NIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BET surface area (m² g⁻¹)</td>
<td>239.17</td>
</tr>
<tr>
<td></td>
<td>Pore volume (cm³ g⁻¹)</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Pore size (nm)</td>
<td>8.26</td>
</tr>
</tbody>
</table>

* Non-imprinted polymer.
with 5 mL loading volume was lower due to less or incomplete interaction of the analytes with active/binding sites of the extracting material. Highest recoveries for all SPE sorbents were observed at loading volume 10 mL indicating that the analyte was fully bound to the active sites. At loading volume >10 mL, the analyte recoveries for all sorbents decreased probably because the active sites of the sorbents were over saturated and thus additional analyte could not be absorbed with increasing loading volume. Thus, 10 mL was selected as the optimal sample loading volume.

3.2.3. Effect of elution solvent and volume

In this study, methanol was chosen as the eluting solvent as eluent since it has the properties of having stronger hydrogen bond and high permeability of analyte in methanol that may induce efficient elution (Cacho et al., 2009; Ju et al., 2007). The addition of a small percentage of acetic acid (1–15%) in the mixture was applied in order to overcome strong interactions between analyte and the MIP and thus enhancing the enrichment factor (He et al., 2007).

Pure methanol (0% acetic acid) was tested in order to confirm that acetic acid played an important role in desorbing quinalphos from the MIP an NIP sorbent in elution solvent. The results showed that the addition of acetic acid increased the analyte recovery (Fig. 4). The most likely explanation was that acetic acid competed with quinalphos for the functional group in the binding sites. However, solvents with relatively high percentage (10% and 15%) of acetic acid apparently tend to decrease the analyte recovery. Thus, 5% of acetic acid in methanol was selected as optimal elution solvent for MIP-SPE.

To optimize the eluting volume, different volumes (3, 6, 10 and 15 mL) of an elution solvent were used. The results both MIP-SPE and NIP-SPE showed that by increasing the solvent volume from 3 mL to 6 mL, the recovery of quinalphos extracted increased from 82% to 98%. However, when the elution volume was increased from 10 mL to 15 mL, the analyte recovery decreased from 71% to 53%. Thus, 6 mL was selected as optimum elution volume for MIP-SPE methods.

3.3. Method validation

The analytical performance of the developed MIP-SPE method was validated through the determination of linearity, sensitivity, repeatability, limits of detection (LOD) and limit of quantification (LOQ) using the optimized MIP-SPE conditions prior to real sample analysis.

The concentration range tested for diazinon, quinalphos and chlorpyrifos were 3–200 μg L⁻¹, 1–250 μg L⁻¹, 4–225 μg L⁻¹, respectively. Each analyte exhibited good linearity with coefficient of determination (r²) ranging from 0.997 to 0.999 (Table 3). The analyte recoveries obtained ranged from 91.5% to 101.04%. It was noted that the recovery for quinalphos was higher compared to the other two analytes. This is expected as quinalphos was used as template which produces selectivity in terms of shape and functional group. Nevertheless, the other two analytes (diazinon and chlorpyrifos) were also successfully extracted because of the similarity of analogue and chemical structures with the template. The method also showed low limits of detection (S/N = 3) obtained for all analytes in the range of 0.83–2.80 μg L⁻¹ and good repeatability with RSD ranging from 0.79% to 1.47%. The maximum residue limits (MRLs) set by Codex Alimentarius Commission (CAC) in grapes and apple for diazinone are 0.7 and 0.1 mg kg⁻¹, respectively while for chlorpyrifos in grapes and apple are 1 mg kg⁻¹ for each (MRLs for Spices, http://www.codexalimentarius.net/pictures/data/MRLSpices_e.pdf). For quinalphos, the MRLs set by Japan food chemical research foundation in grapes and apple are 0.8 and 0.02 mg kg⁻¹, respectively (The Japan Food Chemical Research Foundation, http://www.m5.ws001.squarespace.com/japan/ foundation/agdtp.php?alq_in=17700). The obtained LOD values are much lower compared to the respective MRL values. The excellent results indicated that the developed MIP-SPE method successfully extracted the selected analyte from water sample.

3.4. Applications to fruit samples

The potential of the developed MIP-SPE method for the selective sample clean-up were investigated in the analysis of selected fruit samples, namely apples and grapes. The fruit samples were extracted by using the optimized MIP-SPE procedure. It was found that none of the analytes were detected in the fruit samples (Fig. 5a). This was probably because the OPPs were absent or present at ultra-trace levels in the fruit samples. Thus, in order to determine the accuracy of the method, the samples were spiked at 25 μg L⁻¹ and the extraction performances were evaluated (Fig. 5b). Extraction with NIP-SPE and commercial C18-SPE was performed on fruit samples for comparison. The percentage recovery and RSD of the spiked samples for grape and green apple samples are listed in Table 4. Excellent recoveries were obtained for spiked sample using MIP-SPE ranging from 89.7% to 99.7% with RSDs ranging from 1.3% to 3.1%. These results are anticipated since the MIP-SPE is more selective towards the analytes due to the presence of template imprinted in the polymer and exhibited molecular recognition properties towards other structurally related compounds. In addition, it could also be due to the more porous structure of the imprinted polymer which enables better interactions with the analytes.

### Table 3

Validation parameters for molecularly imprinted polymer solid phase extraction.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Concentration range (μg L⁻¹)</th>
<th>r²</th>
<th>LODa (μg L⁻¹)</th>
<th>LOQa (μg L⁻¹)</th>
<th>RSDb (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>3–200</td>
<td>0.997</td>
<td>1.68</td>
<td>5.99</td>
<td>1.47</td>
<td>97.7</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>1–250</td>
<td>0.999</td>
<td>0.83</td>
<td>2.77</td>
<td>0.79</td>
<td>101.0</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>4–225</td>
<td>0.997</td>
<td>2.80</td>
<td>9.34</td>
<td>1.00</td>
<td>91.5</td>
</tr>
</tbody>
</table>

a LOD = limit of detection; LOQ = limit of quantitation; RSD = relative standard deviation.
Table 4
Recovery (%) and precision (relative standard deviation, RSD) of organophosphorus pesticides (OPPs) in grape and green apple samples.

<table>
<thead>
<tr>
<th>OPP</th>
<th>MIP-SPE</th>
<th>NIP-SPE</th>
<th>C18-SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (n=3)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>Grape</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>95.6</td>
<td>1.62</td>
<td>66.2</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>99.7</td>
<td>1.28</td>
<td>64.8</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>91.0</td>
<td>2.79</td>
<td>59.39</td>
</tr>
<tr>
<td>Green apple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>93.8</td>
<td>2.95</td>
<td>62.5</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>96.5</td>
<td>1.46</td>
<td>59.8</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>89.7</td>
<td>3.12</td>
<td>57.1</td>
</tr>
</tbody>
</table>

* MIP-SPE: molecularly imprinted polymer solid phase extraction; NIP-SPE: non-imprinted polymer solid phase extraction.

Table 5
Recovery study and comparison of statistical method (t-test) and p-value t-test of (MIP-SPE and C18-SPE) and MIP-SPE and NIP-SPE.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recoveries (%)</th>
<th>MIP-SPE</th>
<th>C18-SPE</th>
<th>p-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>97.7</td>
<td>89.0</td>
<td>0.0109</td>
<td>97.7</td>
<td>70.0</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>101.0</td>
<td>90.4</td>
<td>0.0103</td>
<td>101.0</td>
<td>66.5</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>91.5</td>
<td>91.5</td>
<td>0.0218</td>
<td>91.5</td>
<td>62.7</td>
</tr>
</tbody>
</table>

* MIP-SPE: molecularly imprinted polymer solid phase extraction; NIP-SPE: non-imprinted polymer solid phase extraction.

The results of spiked samples for NIP-SPE showed lower analyte recoveries ranging from 57.1% to 66.2% with RSDs < 6% as compared with those for MIP-SPE. High percent recovery was not achieved probably due to the lower energy and non-specific interactions between analyte and polymer as there was no predefined interaction sites which caused weak retention inside the polymer.

The analytes recovery and RSD results obtained for fruit samples using C18-SPE were in the range of 84.3–90.5% and RSDs ranging from 2.0% to 4.2%, respectively. The method showed higher recoveries than those for NIP-SPE method, this was probably due to the dipole–dipole interactions between the sorbent and analyte since both were non-polar compounds. However, the C18-SPE recoveries for both samples were slightly lower than those for the developed MIP-SPE method probably due to the matrix effect from sample and the sorbent lacked selectivity towards the analyte (Cacho et al., 2009; Zhu et al., 2005). The recoveries for both NIP-SPE and C18-SPE were found to be lower those for MIP-SPE.

3.5. Statistical comparison of SPE methods

A statistical hypothesis test namely t-test was used to determine the possibility that the MIP-SPE was significantly different from NIP-SPE and C18-SPE methods. In order to compare these methods, the concentration of 25 μg L⁻¹ for each analyte was used and the recovery and p-value were reported in Table 5. The validation recoveries of MIP-SPE, commercial C18-SPE and NIP-SPE were in the range of 91.5–101.0%, 89.0–91.5%, and 62.7–69.9%, respectively, and the p-values for the comparison of MIP-SPE and C18-SPE for independent t-test were between 0.01 and 0.02. Meanwhile, the p-values for the comparison of MIP-SPE and NIP-SPE for independent t-test were between 0.0001 and 0.0103. Since the p-values for both comparison was less than α = 0.05, the null hypothesis is rejected. Hence, it showed that the sets of data for MIP-SPE were significantly different for both C18-SPE and NIP-SPE methods.

4. Conclusion

The results of the synthesized molecularly imprinted polymers (MIPs) through non-covalent imprinting polymerization indicate their fast, selective, inexpensive and efficient extraction of OPPs (diazinon, quinalphos and chlorpyrifos) in fruit samples. The limits of detection are quite good and with no extra peak in the HPLC chromatogram indicating the selective nature of MIP-SPE method. Moreover, the low cost and excellent stability of MIPs make them most promising synthetic materials for the extraction of analytes in fruit samples as compared with NIP-SPE and a commercial sorbent (C18-SPE).

Acknowledgement

The authors would like to thank UniversitiTeknologi Malaysia for facilitations and the Ministry of Higher Education Malaysia (MOHE) and the Ministry of Science, Technology and Innovation (MOSTI) for financial supports through vote numbers 78477 and
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