Rapid and Direct Microextraction of Pesticide Residues from Rice and Vegetable Samples by Supramolecular Solvent in Combination with Chemometrical Data Processing

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Abstract

In this work, a rapid, simple, and environmentally friendly method has been proposed for direct supramolecular microextraction of four organophosphate insecticides (ethion, phosalone, diazinon, chlorpyrifos) and an isothiazolidine acaricide (hexythiazox) in agricultural product samples prior to their determination by high-performance liquid chromatography-ultraviolet spectroscopy. These five target pesticides have been selected as models in combination with chemometrical optimization processing due to their high consumption in rice, cucumber, and tomato samples for pest control. Method is based on the extraction of pesticide residues from homogenized food sample in an aqueous media containing some tetrahydrofuran (THF) and decanoic acid (DeA). Effects of the experimental parameters, including THF volume, DeA content, salt concentration (as a measure of salting-out effect), and pH on extraction recoveries (ERs) and enrichment factors (EFs) were investigated and, then, the significant variables were optimized using central composite design (CCD) as chemometrical processing. At optimum conditions, this method has a linear response over the ranges of 0.10 to 1500 \(\mu\)g kg\(^{-1}\) for target analytes. Limits of detection (LOD) of this method were found to be in the range of 0.05 to 0.20 \(\mu\)g kg\(^{-1}\). Also, relative standard deviation (RSD) of the method was in the range of 3.45 to 12.27% and the enrichment factors ranged from 102- to 178-fold. The method was applied successfully for analysis of the pesticides in agricultural product samples.

Keywords Central composite design · Direct supramolecular microextraction · High-performance liquid chromatography-ultraviolet spectroscopy · Pesticide residue · Rice and vegetable samples

Highlights
• A rapid, simple and environmental friendly method for preconcentration and separation of trace pesticide residues has been proposed.
• Method is based on the extraction of pesticide residues from homogenized food samples by direct supramolecular solvent.
• Several effective parameters on extraction of pesticides were studied and optimized using a chemometrical optimization processing.
• RMSSs-HPLC/UV method is applied successfully to simultaneous extraction of five pesticides in tomato, rice, and cucumber samples.

Introduction

Pesticides are used on a large scale for agricultural purposes (Dorr et al. 2007; Ritter et al. 2006) because of their powerful bioactivity (Al-Degs et al. 2009) and their susceptibility to insect and disease attacks (Ridgway et al. 2007). Application of pesticides in agriculture helps to obtain higher quality vegetables and fruits by spending a lower cost. However, use of pesticides leaves some residues in food, which threaten overall population health and the remained residual pesticides are of noticeable importance in food quality evaluation (Goto et al. 2003).

Most pesticide residues occur in food as a result of the direct application of a pesticide to a crop or farm animal or the post-harvest treatments of food commodities, such as grains to prevent pest attack (Denis and Stephen 2004). In the world, alarming levels of pesticides have been reported in air, water, soil, as well as in foods and biological materials (Lambropoulou and Albanis 2007). Hence, there is an increasing demand for developing rapid, reliable, and inexpensive
methods for the determination of such contaminations in (Ridgway et al. 2007) food and vegetable samples.

The monitoring of pesticide residues is important to ensure not only food safety but also compliance with good manufacturing practices (Lucía et al. 2011). However, the complicated matrix of the agricultural products may affect the accuracy of the analysis. Hence, the pretreatment before the analysis is very important (Liu et al. 2006). Several methods have been proposed for separation and preconcentration of trace pesticides, including liquid–solid extraction (Cortés et al. 2006); solid-phase extraction (Asperger et al. 2006); Soxhlet extraction (Sanghi and Tewari 2001); liquid-liquid extraction (Britoa et al. 2002; Sabik and Jeannot 1998); solid-phase microextraction (Chai et al. 2008; Sampedro et al. 2000; Tsoutsis et al. 2006); matrix solid-phase dispersion (Abhilash et al. 2007; Hashi et al. 2005; Liu et al. 2005); accelerated solvent extraction (Wang et al. 2010) and microwave-assisted extraction (Barriada-Pereira et al. 2003); magnetic solid-phase extraction (Yu and Yang 2017; Khodadoust et al. 2018; Yu et al. 2018); and nanoparticle-basis extraction (Amiri Pebdani et al. 2016; Talebianpoor et al. 2017). However, some of these methods are time-consuming, some need considerable amounts of organic solvents and some are expensive. Furthermore, most analysis time by these techniques is involved with just sample preparation.

Recently, supramolecular solvents have been used in extraction techniques (Ruiz et al. 2006). Supramolecular solvents are water-immiscible liquids made up of supramolecular assemblies (as reverse micelles of decanoic acid) dispersed in a continuous phase (as THF: water) (José López-Lar et al. assemblies (as reverse micelles of decanoic acid) dispersed in a continuous phase (as THF: water) (José López-Lar et al. 2010) and microwave-assisted extraction (Barriada-Pereira et al. 2003); magnetic solid-phase extraction (Yu and Yang 2017; Khodadoust et al. 2018; Yu et al. 2018); and nanoparticle-basis extraction (Amiri Pebdani et al. 2016; Talebianpoor et al. 2017). However, some of these methods are time-consuming, some need considerable amounts of organic solvents and some are expensive. Furthermore, most analysis time by these techniques is involved with just sample preparation.

This study concentrates on applying rapid and ecofriendly microextraction of five pesticide residues as model from rice and vegetable samples by direct supramolecular solvent in combination with chemometrical data processing prior to HPLC/UV. Effects of the different experimental parameters on extraction recoveries and enrichment factors were investigated and, then, the significant variables were optimized using central composite design (CCD). The objective of this paper was applying a convenient, fast, low cost, and environmentally friendly method that is appropriate for routine control of pesticide contaminants. Also, according to our knowledge, this method has not been applied for measuring of the simultaneous pesticides in rice sample. Furthermore, quantitative performance of the method was evaluated by RMSSs-HPLC/UV.

Materials and Methods

Chemical and Reagents

Standard pesticides of (diazinon, phosalone, and chlorpyrifos) and (ethion and hexthiazox) were purchased from Sigma–
Aldrich (St. Louis, MO, USA) and Chem Service (West Chester, Pennsylvania, USA), respectively. Stock standard solutions were prepared by dissolving 200 μg mL⁻¹ phosalone and 500 μg mL⁻¹ of the other pesticides in methanol. The prepared standard solutions were stored in a freezer at −18 °C. Mixtures of the standard working solutions were prepared weekly in methanol and stored in brown bottles at −18 °C. Methanol was purchased from Romil, LTD (Cambridge, UK). Sodium chloride and hydrochloric acid were purchased from Merck (Germany). Analytical grade tetrahydrofuran was supplied from Fluka (Buchs, Switzerland), and high purity water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All reagents were of analytical grade or above.

Agricultural product samples (tomato, cucumber, and rice) were purchased from local supermarkets (Sari, Iran), randomly.

**Instrumentation**

A KNÄUER HPLC model Smartline with ultra violet (UV) 2500 detector (Berlin, Germany) and a 20-μL sample loop was used for separation and detection of the pesticides. Analyte separation was performed on a reversed phase C18 HPLC Nucleodur column (5 μm, 250 × 4.6 mm, Macherey-Nagel Co., Düren, Germany). Isocratic elution was performed with 80:20 (v/v) methanol: water effluent. Flow rate, injection volume, and UV wavelength were set at 0.7-mL min⁻¹, 20 μL, and 237 nm, respectively. A kitchen homogenizer-blender (Cariton JE-5000, China) and a bench centrifuge device (Sahand azma, Tehran, Iran) were used for sample preparation. Measurement of pH was conducted by a pH meter (WTW, Weilheim, Germany). A 25-mL volumetric flask (110-mm height and 9-mm internal diameter) was used for the RMSSs process. Further, measurement of coacervate volume in the narrow neck of the vial was made by a digital caliper.

**Sample Preparation**

Tomato, cucumber, and rice samples were purchased from local supermarkets, randomly. The vegetable samples were chopped using a kitchen knife and homogenized by a blender. The rice sample was thoroughly powdered by a homogenizer until no visible piece of rice remained in the final sample. The homogenized and powdered samples were stored at −4 °C until used. Scheme 1 shows the sequential steps followed for determination of the pesticides in the agricultural product samples including the microextraction and quantitation steps.

**Reverse Micelle-based Direct Supramolecular Solvents Microextraction Procedure**

First, 300 mg rice or vegetable (tomato, cucumber) samples was introduced in a 25-mL volumetric flask and then, a decanoic acid (100 mg) solution in THF (1.75 mL) was added in sequence. Next, about 22–23 mL 3% (w/v) NaCl solution (pH 2.00 ± 0.01) was added into the volumetric flask and reached the final volume of 25 mL. Spontaneously, supramolecular solvent formed into the bulk solution. The mixture was stirred at 1000 rpm for 10 min to enhance extraction rate of the pesticides. Then, the mixture was centrifuged for 10 min at × 1465g to speed up separation of the coacervate phase from the bulk solution. Finally, volume of the coacervate phase (around 168–183 μL), which was standing at the narrow neck of the volumetric flask, was calculated by measuring its height with a digital caliper. Aliquot of the coacervate was withdrawn using a microsyringe and directly injected into the HPLC-UV system for analysis.
The mentioned agricultural samples were directly added to ternary mixtures of decanoic acid, THF, and water. Always, three phases were observed after extraction and centrifugation of the vegetable and rice samples, which included a solid-phase made up of insoluble matrix components at the bottom, a THF: water solution containing decanoic acid monomers and dissolved matrix components in the middle and a coacervate phase containing the extracted target analytes at the top. At equilibrium, the pesticides distributed among these three phases, although high solubility of the pesticides in the coacervate phase, compared with their solubility in THF: water, greatly favored partition of the analytes to the coacervate phase (Prieto et al. 2008).

**Calculation of the Phase Volume Ratio (PVR)**

By measuring internal diameter of the narrow neck of the volumetric flask and height of the formed coacervate phase by a digital caliper, volume of the separated coacervate phase was calculated using the following equation:

$$V_c = (2r^2)h$$

(1)

$$PVR = \left( \frac{V_0}{V_c} \right)$$

(2)

where $r$ and $h$ are internal radius of the narrow volumetric flask neck and height of the formed coacervate phase, respectively. Ratio of the initial volume of the sample ($V_0$) to volume of the separated coacervate phase ($V_c$), i.e., PVR, is a measure of the enrichment factor.

To calculate EF and ER, it is necessary to determine concentration of the analyte in the coacervate and aqueous phases. By measuring analyte concentration in the coacervate phase using a chromatographic method, concentration of the analyte in the aqueous phase can be obtained through Eq. (3), with respect to mass balance.

$$[A]_0 V_0 = [A]_{aq} V_{aq} + [A]_c V_c$$

(3)

where $[A]_0$, $[A]_{aq}$, and $[A]_c$ are the initial concentration of the analyte, concentration of the analyte in the aqueous phase, and the coacervate phase, respectively. Also, $V_{aq}$ is the volume of aqueous phase after RMSSs procedure.

**Calculation of Enrichment Factor and Extraction Recovery**

EF was considered to be ratio of the analyte concentration dissolved in the coacervate phase ([A]c) to its concentration in the initial aqueous sample ([A]0) (Khodadoust et al. 2013; Rezaee et al. 2006):

$$EF = \frac{[A]_c}{[A]_0} = \frac{\text{mol} [A]_c \times V_0}{\text{mol} [A]_0 \times V_c} = \frac{\text{mol} [A]_c}{\text{mol} [A]_0} \times PVR$$

(4)

Calculation of $[A]_c$, for each extracted analyte was conducted through chromatographic method and calibration graphs. Percentage of the analyte extracted into the coacervative phase was defined as ER (Eq. (5)). In this equation, $V_c$ and $V_{aq}$ are volumes of the coacervative phase and the aqueous phase, respectively (Fattahi et al. 2007).

$$ER = \left( \frac{[A]_c V_c}{[A]_0 V_{aq}} \right) \times 100 = EF \times \left( \frac{V_c}{V_{aq}} \right) \times 100$$

(5)

**Result and Discussion**

**Coacervate Description**

When decanoic acid dissolves in THF, it can form reverse micelles. Adding water to decanoic acid-THF binary system can dissolve the micellar aggregates partially and facilitate micelle-micelle interactions. As an outcome, sizes of the formed supramolecular aggregates increase and the aggregates separate from the bulk solution as a liquid phase that is immiscible. This phase is known as coacervate. Therefore, water, which is not able to dissolve decanoic acid, is the coacervation inducer agent. At microscopic level, coacervate structure contains spherical droplets consisted of different numbers of reverse micelles that are dispersed in the continuous phase of THF-water. Since the generated reverse micelles are products of protonated decanoic acid (pKa = 4.8 ± 0.2), pH < 4 is necessary to obtain the coacervate phase. The coacervate supramolecular aggregates exhibit two interaction types for solute extraction. These interactions are van der Waals interactions of the hydrocarbon chains of decanoic acid molecules and hydrogen bonding of the micellar core. Coacervate volume depends on concentrations of decanoic acid and THF.

**Experimental Design**

Tendency of an analyte towards a specific micelle aggregate relies on natures of the analyte and the micelle. The reason is that functional groups of the monomers lead to various micelle micro-mediums with slight differences in their physicochemical properties. These properties can be tuned to promote affinity of the aggregates towards certain analytes, selectively. Consequently, efficiency of coacervative microextraction can be justified by altering various physicochemical parameters that influence
Table 1 Variables and their levels employed in a central composite design for the evaluation of the efficiency of extraction using RMSSs microextraction

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of THF (mL)</td>
<td>− α (−2) 1.00</td>
</tr>
<tr>
<td>Decanoic acid weight (mg)</td>
<td>−1 50</td>
</tr>
<tr>
<td>Salt concentration (% w/v, NaCl)</td>
<td>0 2</td>
</tr>
<tr>
<td>pH</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 2 The actual experimental parameters in four-variable, five-level CCD for study and optimization of target pesticides

<table>
<thead>
<tr>
<th>SET</th>
<th>A volume of THF (mL)</th>
<th>B Decanoic acid (mg)</th>
<th>C Salt concentration (% w/v, NaCl)</th>
<th>D pH</th>
<th>Coacervate volume (μL)</th>
<th>Sum of extraction recovery for five pesticides (response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75 (−1)</td>
<td>100 (−1)</td>
<td>2 (−1)</td>
<td>1.00 (−1)</td>
<td>120</td>
<td>6.01 × 10⁶</td>
</tr>
<tr>
<td>2</td>
<td>3.25 (+1)</td>
<td>100 (−1)</td>
<td>2 (−1)</td>
<td>1.00 (−1)</td>
<td>199</td>
<td>8.36 × 10⁶</td>
</tr>
<tr>
<td>3</td>
<td>1.75 (−1)</td>
<td>200 (+1)</td>
<td>2 (−1)</td>
<td>1.00 (−1)</td>
<td>330</td>
<td>9.02 × 10⁶</td>
</tr>
<tr>
<td>4</td>
<td>3.25 (+1)</td>
<td>200 (+1)</td>
<td>2 (−1)</td>
<td>1.00 (−1)</td>
<td>390</td>
<td>1.15 × 10⁷</td>
</tr>
<tr>
<td>5</td>
<td>1.75 (−1)</td>
<td>100 (−1)</td>
<td>6 (+1)</td>
<td>1.00 (−1)</td>
<td>140</td>
<td>2.59 × 10⁶</td>
</tr>
<tr>
<td>6</td>
<td>3.25 (+1)</td>
<td>100 (−1)</td>
<td>6 (+1)</td>
<td>1.00 (−1)</td>
<td>207</td>
<td>9.01 × 10⁶</td>
</tr>
<tr>
<td>7</td>
<td>1.75 (−1)</td>
<td>200 (+1)</td>
<td>6 (+1)</td>
<td>1.00 (−1)</td>
<td>320</td>
<td>1.07 × 10⁷</td>
</tr>
<tr>
<td>8</td>
<td>3.25 (+1)</td>
<td>200 (+1)</td>
<td>6 (+1)</td>
<td>1.00 (−1)</td>
<td>425</td>
<td>1.28 × 10⁷</td>
</tr>
<tr>
<td>9</td>
<td>1.75 (−1)</td>
<td>100 (−1)</td>
<td>2 (−1)</td>
<td>2.00 (+1)</td>
<td>135</td>
<td>2.29 × 10⁷</td>
</tr>
<tr>
<td>10</td>
<td>3.25 (+1)</td>
<td>100 (−1)</td>
<td>2 (−1)</td>
<td>2.00 (+1)</td>
<td>170</td>
<td>3.33 × 10⁷</td>
</tr>
<tr>
<td>11</td>
<td>1.75 (−1)</td>
<td>200 (+1)</td>
<td>2 (−1)</td>
<td>2.00 (+1)</td>
<td>310</td>
<td>1.28 × 10⁷</td>
</tr>
<tr>
<td>12</td>
<td>3.25 (+1)</td>
<td>200 (+1)</td>
<td>2 (−1)</td>
<td>2.00 (+1)</td>
<td>420</td>
<td>5.05 × 10⁷</td>
</tr>
<tr>
<td>13</td>
<td>1.75 (−1)</td>
<td>100 (−1)</td>
<td>6 (+1)</td>
<td>2.00 (+1)</td>
<td>145</td>
<td>2.21 × 10⁷</td>
</tr>
<tr>
<td>14</td>
<td>3.25 (+1)</td>
<td>100 (−1)</td>
<td>6 (+1)</td>
<td>2.00 (+1)</td>
<td>183</td>
<td>1.43 × 10⁷</td>
</tr>
<tr>
<td>15</td>
<td>1.75 (−1)</td>
<td>200 (+1)</td>
<td>6 (+1)</td>
<td>2.00 (+1)</td>
<td>295</td>
<td>1.24 × 10⁷</td>
</tr>
<tr>
<td>16</td>
<td>3.25 (+1)</td>
<td>200 (+1)</td>
<td>6 (+1)</td>
<td>2.00 (+1)</td>
<td>275</td>
<td>5.09 × 10⁶</td>
</tr>
<tr>
<td>17</td>
<td>1.00 (−2)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>190</td>
<td>1.70 × 10⁷</td>
</tr>
<tr>
<td>18</td>
<td>4.00 (+2)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>345</td>
<td>9.60 × 10⁶</td>
</tr>
<tr>
<td>19</td>
<td>2.50 (0)</td>
<td>50 (−2)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>86</td>
<td>1.16 × 10⁷</td>
</tr>
<tr>
<td>20</td>
<td>2.50 (0)</td>
<td>250 (+2)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>353</td>
<td>6.80 × 10⁶</td>
</tr>
<tr>
<td>21</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>0 (−2)</td>
<td>1.50 (0)</td>
<td>260</td>
<td>1.11 × 10⁷</td>
</tr>
<tr>
<td>22</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>8 (+2)</td>
<td>1.50 (0)</td>
<td>290</td>
<td>1.10 × 10⁷</td>
</tr>
<tr>
<td>23</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>0.50 (−2)</td>
<td>235</td>
<td>8.00 × 10⁶</td>
</tr>
<tr>
<td>24</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>2.50 (+2)</td>
<td>330</td>
<td>1.49 × 10⁷</td>
</tr>
<tr>
<td>25</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>298</td>
<td>9.09 × 10⁶</td>
</tr>
<tr>
<td>26</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>230</td>
<td>1.07 × 10⁷</td>
</tr>
<tr>
<td>27</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>276</td>
<td>1.01 × 10⁷</td>
</tr>
<tr>
<td>28</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>275</td>
<td>1.02 × 10⁷</td>
</tr>
<tr>
<td>29</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>265</td>
<td>1.04 × 10⁷</td>
</tr>
<tr>
<td>30</td>
<td>2.50 (0)</td>
<td>150 (0)</td>
<td>4 (0)</td>
<td>1.50 (0)</td>
<td>255</td>
<td>1.20 × 10⁷</td>
</tr>
</tbody>
</table>
Salt concentration, and pH were optimized using a five-level four-factor central composite design. After determining ranges of the RMSSs variables, 30 experiments were designed to find interactions between the four variables mentioned above. Table 1 presents coded and non-coded values of the experimental variables. Design of experiment along with the enrichment factor is given in Table 2. The relationship between the independent variables and the response was calculated by a second-order polynomial equation. To find the most important effects and interactions, analysis of variance (ANOVA) was carried out using the software package of Design Expert 7 (Montgomery 2001).

Model $F$ value of Eq. (6) was obtained as 62.97, which implies its significance. According to Eq. (6), this model includes three main effects, two curvature impacts and three two-factor interaction effects. A model with such large $F$ value has only 0.01% chance of being resulted from noises. “Prob > $F$” of the model was calculated to be below 0.0500, which demonstrates that the model’s $A$, $B$, $D$, $AD$, $BD$, and $A^2$ terms are significant. “Prob > $F$” values above 0.1000 outline insignificant model terms. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. “Lack of fit $F$ value” of 1.26 declares insignificant lack of fit, with respect to the pure error. Such “lack of fit $F$ value” is associated with 42.86% chance of being due to noise. It should be noted that insignificant lack of fit is a positive point.

Table 3 ANOVA results of the Design Expert 7 for studied response

<table>
<thead>
<tr>
<th>Response</th>
<th>Probability for model</th>
<th>$R^2$</th>
<th>Adj. $R^2$</th>
<th>Pred. $R^2$</th>
<th>Adeq. precision</th>
<th>SD</th>
<th>CV</th>
<th>PERESS</th>
<th>Probability for lack of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chromatographic peak area</td>
<td>&lt; 0.0001</td>
<td>0.9600</td>
<td>0.9447</td>
<td>0.9126</td>
<td>31.74</td>
<td>1.0450 × 10⁶</td>
<td>9.470</td>
<td>5.010 × 10¹³</td>
<td>0.4286</td>
</tr>
</tbody>
</table>

$a$ $R^2$, determination coefficient  
$b$ Adj. $R^2$, adjusted $R^2$  
$c$ Pred. $R^2$, predicted $R^2$  
$d$ Adeq. precision: adequate precision  
$e$ SD, standard deviation  
$f$ CV, coefficient of variation  
$g$ PERESS, predicted residual error sum of squares

Total chromatographic peak area (response)

$$= 1 \cdot 0.08 \times 10^7 - 1 \cdot 44 \times 10^6 A - 1 \cdot 23 \times 10^6 B + 2 \cdot 20 \times 10^6 D - 6 \cdot 36 \times 10^4 AB - 2 \cdot 91 \times 10^6 AD - 3 \cdot 51 \times 10^6 BD + 6 \cdot 41 \times 10^5 A^2 - 3 \cdot 72 \times 10^5 B^2 \quad (6)$$

Evaluation of the fitted model is always necessary to ensure that the model offers an acceptable estimation of the real system and confirm that none of the least-squares

Table 4 Coefficient and ANOVA output for the proposed model for the evaluation of the extraction efficiency of target pesticides

<table>
<thead>
<tr>
<th>Response</th>
<th>Source</th>
<th>Coefficient estimate</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chromatographic peak area</td>
<td>Model</td>
<td>–</td>
<td>7</td>
<td>5.51 × 10¹⁴</td>
<td>62.97</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>1.08 × 10⁷</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>A—volume of THF (mL)</td>
<td>−1.43 × 10⁶</td>
<td>1</td>
<td>4.94 × 10¹³</td>
<td>45.22</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>B—decanoic acid weight (mg)</td>
<td>−1.23 × 10⁶</td>
<td>1</td>
<td>3.65 × 10¹³</td>
<td>33.35</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>D—pH</td>
<td>2.18 × 10⁶</td>
<td>1</td>
<td>1.15 × 10¹⁴</td>
<td>105.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>−6.36 × 10⁴</td>
<td>1</td>
<td>6.49 × 10¹⁰</td>
<td>0.06</td>
<td>0.8100</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>−2.91 × 10⁶</td>
<td>1</td>
<td>1.36 × 10¹⁴</td>
<td>124.18</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>−3.51 × 10⁶</td>
<td>1</td>
<td>1.97 × 10¹⁴</td>
<td>179.98</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>$A^2$</td>
<td>6.41 × 10⁵</td>
<td>1</td>
<td>1.17 × 10¹⁰</td>
<td>10.68</td>
<td>&lt; 0.0037</td>
</tr>
<tr>
<td></td>
<td>$B^2$</td>
<td>−3.72 × 10⁵</td>
<td>1</td>
<td>3.93 × 10¹²</td>
<td>3.59</td>
<td>&lt; 0.0718</td>
</tr>
</tbody>
</table>
regression assumptions has been disaffirmed. Statistical significance of the model was assessed through ANOVA. The results are listed in Tables 3 and 4. Quality of the fitted polynomial model was expressed by coefficients of determination ($R^2$), adjusted $R^2$ (adj. $R^2$) and adequate precision (pred. $R^2$). $R^2$ is indicative of variations in the model's results around the average value and is equal to 0.9600. In addition to $R^2$, adjusted $R^2$ was considered for the model's
number of terms. Adjusted $R^2$ would equal to 0.9447 if the additional terms do not add value to the model. Adjusted $R^2$ decreases, as the number of model terms increases. Sufficient precision is indicated by signal-to-noise ratio, which compares the predicted range of values at the design points to mean prediction error. Signal-to-noise ratios above 4 signify sufficiency of model discrimination. In the case of this study, this ratio is 31.74.

Response of the model was mapped versus two experimental factors while the other two factors were kept constant at their central levels. According to Fig. 1a and Table 4 ($P$ value and $F$ ratios of the ANOVA data), there is no interaction between volume of THF and weight of decanoic acid. Decanoic acid affects generation of reverse micelle and volume of the extraction phase. Therefore, it influences the peak area. Effect of decanoic acid weight on the peak area was studied in the range of 50 to 250 mg. Coacervation phase was easily achieved using a lower weight of decanoic acid and less THF volume.

Figure 1b shows effects of THF volume and pH on extraction of the analytes. The outcomes indicate that the response increases with an increase in pH and decrease of THF volume. According to this figure and Table 4, there is a significant interaction between volume of THF and pH.

In Fig. 1c, there is a combined effect of decanoic acid weight and pH. As it can be seen, the signal decreases with simultaneous increase of decanoic acid weight and pH, at constant values of THF volume, and salt concentration. According to this figure and Table 4, interaction between decanoic acid weight and pH is significant.

Coacervation just takes place in solutions that include protonated decanoic acid molecules ($pK_a = 4.8 \pm 0.2$) (Fonseca et al. 2008). Therefore, pH effect was investigated by changing pH from 0.50 to 2.50. The obtained results declared that the maximal extraction efficiencies can be obtained at pH values around 2.00 (Figure 1c). Consequently, pH = 2.00 was chosen to extract the pesticides from the sample solutions.

Variance analysis of Eq. (6) and the optimization function of Design Expert software approved that salt concentration has affected about 3% ($w/v$) on the extraction process. However, at higher salt concentrations, solution density increases; therefore, reverse micelle coacervation phase generates slightly.

### Optimum Conditions

RSM and the optimization function of Design Expert software were applied to predict optimal conditions of extraction and preconcentration of the target pesticides in the evaluated experimental ranges. They showed that the highest value of the response can be obtained at...
THF volume of 1.75 mL, decanoic acid weight of 100 mg, NaCl concentration of 3% (w/v), and pH of 2.00. Extraction recoveries, enrichment factors, coacervate volumes, and predicted and experimental response values are presented in Table 5. Comparison between the expected and experimental response values (Table 5) for water samples showed excellent agreement between them, which implies that the empirical model derived from application of RSM and the optimization function can be used to adequately describe the relationship between the variables and the response in extraction and preconcentration of the pesticides.

### Influence of Real Samples Matrix on Coacervative Efficiency

The matrix effect of target agricultural product samples on the coacervative efficiency, the experimental and predicted response values investigated and reported in Table 5. For this purpose, EF, ER, and total experimental chromatographic responses of tomato, rice, and cucumber samples with water sample were compared. As Table 5 exhibits, on the one hand, obtained experimental responses for tomato, rice, and cucumber samples were different from the water sample, and on the other hand, there

<table>
<thead>
<tr>
<th>Table 5 Analytical performances of the proposed method for the five target pesticides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pesticides</strong></td>
</tr>
<tr>
<td>Ethion</td>
</tr>
<tr>
<td>Phosalone</td>
</tr>
<tr>
<td>Diazinon</td>
</tr>
<tr>
<td>Hexythiazox</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
</tr>
</tbody>
</table>

a Relative standard deviation at concentration of 0.20 μg kg⁻¹ phosalone and 0.50 μg kg⁻¹ of the other pesticides

### Table 6 Analytical performance of RMSSs-HPLC/UV for simultaneous analysis of target pesticides in real samples

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Cucumber sample</th>
<th>Rice sample</th>
<th>Tomato sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Added (μg kg⁻¹)</strong></td>
<td><strong>Found (μg kg⁻¹)</strong></td>
<td><strong>Recovery (%) ± RSD (n = 3)</strong></td>
<td><strong>Added (μg kg⁻¹)</strong></td>
</tr>
<tr>
<td>Ethion</td>
<td>0.00</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.24</td>
<td>96.0 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>9.70</td>
<td>97.0 ± 6.4</td>
</tr>
<tr>
<td>Phosalone</td>
<td>0.00</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.19</td>
<td>95.0 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.70</td>
<td>94.0 ± 11.3</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.00</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.24</td>
<td>96.0 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>10.56</td>
<td>105.6 ± 8.4</td>
</tr>
<tr>
<td>Hexythiazox</td>
<td>0.00</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.23</td>
<td>92.0 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>9.24</td>
<td>92.4 ± 6.7</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.00</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.21</td>
<td>84.0 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>8.86</td>
<td>88.6 ± 9.1</td>
</tr>
</tbody>
</table>

a Not detected
were some differences between the predicted response value by the model and the experimentally measured response values for the tomato, rice, and cucumber samples. The reason refers to influences of the higher volume of formed coacervate phase in tomato, rice, and cucumber samples compared to water sample. This issue led to the dilution of pesticides concentration in coacervate phase, resulting in total experimental chromatographic peak area of target pesticides decreased relative to the predicted values. An interesting point in the tomato, rice, and cucumber samples was that the reduction of total chromatographic responses and EFs related to the greater volumes of produced coacervate phases, not the matrix effect of tomato, rice, and cucumber samples because pesticides recoveries in this samples have not been significantly decreased compared to water sample.

**Analytical Performance**

Under the optimum conditions, analytical performance of RMSSs-HPLC/UV for simultaneous determination of the five target pesticides was evaluated and the results are summarized in Table 6. The limit of detections (LODs), based on signal-to-noise ratio (S/N) of 3 ranged from 0.05 to 0.20 μg kg⁻¹. The limit of quantification (LOQs), based on signal-to-noise ratio (S/N) of 10 ranged from 0.17 to 0.65 μg kg⁻¹. Intraday (n = 3) and interday precisions were obtained by extracting samples at concentration level of 0.20 μg kg⁻¹ phosalone and 0.50 μg kg⁻¹ of the other pesticides. The relative standard deviation (RSD%) did not exceed 10.41 and 12.27% for interday and intraday precisions, respectively. The obtained calibration curves illustrated satisfactory linearity in the ranges of 0.25 to 800 μg kg⁻¹ for ethion, 0.10 to 500 μg kg⁻¹ for phosalone, 0.25 to 1500 μg kg⁻¹ for diazinon, 0.25 to 100 μg kg⁻¹ for hexythiazox, and 0.25 to 1000 μg kg⁻¹ for chlorpyrifos. Also, coefficients of determination (r²) for all analytes were over 0.9924.

**Application of the Method to Real Samples**

RMSSs-HPLC/UV was applied for determination of the five target pesticides as model in rice, tomato, and cucumber samples. Analysis of the samples and their extraction recovery values were evaluated in triplicate (Table 7). Though various kinds of rice, tomato, and cucumber samples were analyzed, only the results related to one kind of each sample are reported in Table 7. In the rice sample, 0.62 μg kg⁻¹ diazinon was detected. It should be noted that concentrations of the five pesticides in the analyzed tomato and cucumber samples were below detection limit of the approach. The proposed analytical method was validated by the standard addition method. Based on this standard method, presences of mentioned pesticides in target samples were confirmed by spiking pesticides at the different concentration levels. Figure. 2a, b, c shows the chromatograms obtained for the tomato, rice, and cucumber samples before and after pesticide spiking. This study led to a satisfactory robustness by achieving recoveries in the range of 84.0 to 105.6%.

**Comparison of RMSSs–HPLC/UV with Other Extraction Methods**

Extraction and determination of the target pesticides existing in tomato, rice, and cucumber samples through application of RMSSs in combination with HPLC-UV detection is compared with some other methods in Table 8. RSD values of the proposed method are similar to the other methods. Apparently, performance of RMSSs-HPLC/UV is superior to the other methods, with respect to linear range, solvent volume, amount of the sample, and sample preparation time. So that, linear range is wide (0.1–1500 μg kg⁻¹), solvent volume is very low (1.75 mL), extraction time is relatively short (20 min), and a small amount of the sample (0.3 g) is required in the RMSSs-HPLC/UV method. RMSSs–HPLC/UV method requires a very low amount of organic solvent compared to other extraction methods, and also when a small amount of the sample is available, this method can detect target analytes with a high enrichment factor. Furthermore, RMSSs–HPLC/UV is rapid; it does not require designing a specific tool or vial for extraction of the target analytes and is easily accomplished using a volumetric flask that is available in almost all laboratories.
Table 8  Comparison of RMSS–HPLC/UV with other extraction methods for determination of pesticides in agricultural product samples

<table>
<thead>
<tr>
<th>Methods</th>
<th>LOD$^a$ (μg kg$^{-1}$)</th>
<th>Linear range (μg kg$^{-1}$)</th>
<th>RSD$^b$ (%)</th>
<th>Extractant volume (mL)</th>
<th>Extraction time (min)</th>
<th>Sample (sample amount g))</th>
<th>Analytes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>UASE-DLLME-SFO-HPLC-UV$^c$</td>
<td>1–4</td>
<td>5-800</td>
<td>≤ 9</td>
<td>5 mL + 150 μL</td>
<td>35</td>
<td>Cucumber, Watermelon, Melon, Ribbed melon (1)</td>
<td>Diazinon, fenthion, Phosalone Chloropyrifose</td>
<td>Pirashheb et al. (2013)</td>
</tr>
<tr>
<td>SBSE-GC-TSD$^e$</td>
<td>0.0012–0.15</td>
<td>0.05–50</td>
<td>5–31</td>
<td>–</td>
<td>65</td>
<td>Cucumber and potato (10)</td>
<td>Momocrotophos, phorate, dimethoate, parathion-methyl, malathion, fenitrothion, fenthion, chlorpyrifos, parathion, methidathion, triazophos, ethion</td>
<td>Liu et al. (2005)</td>
</tr>
<tr>
<td>LLE-GC-FID (LVI)$^f$</td>
<td>410–2250</td>
<td>–</td>
<td>&lt;10</td>
<td>5</td>
<td>–</td>
<td>Eggplant, lettuce, pepper, cucumber, and tomato (25)</td>
<td>Dimethoate, chlorpyrifos, diazinon, fenitrothion, malathion, chlorfenvinoxphos, methidathion, fenathion, and tetrachlorvinphos</td>
<td>Cortes et al. (2006)</td>
</tr>
<tr>
<td>MSPE-HPLC-PDA$^g$</td>
<td>0.0290–0.0658</td>
<td>0.2–5.0</td>
<td>2.03–12.01</td>
<td>24</td>
<td>30</td>
<td>Vegetable oils (5)</td>
<td>Tetramethrin, Fenpropatrin, Cypermethrin, Decamethrin, Fenvalerate, Acrinathrin, Permethrin, Bifenthrin, Ethion</td>
<td>Yu et al. (2017)</td>
</tr>
<tr>
<td>RMSS-HPLC/UV$^h$</td>
<td>0.05–0.20</td>
<td>0.1–1500</td>
<td>≤ 12.27</td>
<td>1.75</td>
<td>20</td>
<td>Rice, cucumber, and tomato (0.3 g)</td>
<td>Ethion, Phosalone, Diazinon, Hexythiazox, Chloropyrifos</td>
<td>This work</td>
</tr>
</tbody>
</table>

$^a$ Limit of detection  
$^b$ Relative standard deviation  
$^c$ Ultrasonic assisted solvent extraction-dispersive liquid-liquid microextraction-solidification of floating organic drop high-performance liquid chromatography-ultraviolet detector  
$^d$ Supercritical fluid extraction solid-phase extraction-gas chromatography-mass spectrometer detector  
$^e$ Stir bar sorptive extraction and capillary gas chromatography with thermionic specific detection  
$^f$ Liquid-liquid extraction-gas chromatography-flame ionization detector (large volume injection)  
$^g$ Magnetic solid-phase extraction high-performance liquid chromatography-photo diode array  
$^h$ Reverse micelle-supramolecular solvent microextraction high-performance liquid chromatography-ultra violet detection
Conclusions

Monitoring of pesticide residues in highly consumed agricultural products is important. This study applies direct supramolecular microextraction based on decanoic acid reverse micelle formation, as a rapid, effective, simple, and low-cost method to extract pesticides from tomato, cucumber, and rice agricultural samples. Compared with conventional methods, this method does not require designing a specific tool or vial for extraction of the target analytes and is easily accomplished using a volumetric flask that is available in almost all laboratories. The proposed extraction approach is simple and rapid since it just requires a single extraction step for sample treatment with a few organic solutions containing around 1.75 mL THF and 100 mg decanoic acid. Furthermore, this method does not need clean-up or solvent evaporation, and it consumes very low volumes of organic solvents, which demonstrates environmentally friendly behavior of this method.

Five-level four-factor CCD was used to optimize the factors affecting the extraction process. At optimal conditions, the findings indicated that the method of RMSSs-HPLC/UV can improve extraction and preconcentration of the pesticides efficiently due to its high extraction recovery. This method is suited for routine analysis to determine trace levels of pesticides in agricultural products and environmental samples.

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Compliance with Ethical Standards

Conflict of Interest Setare Gorji declares that she has no conflict of interest. Pourya Biparva declares that he has no conflict of interest. Morteza Bahram declares that he has no conflict of interest. Ghorbanali Nematzadeh declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Publication has been approved by all individual participants.

References


