Determination of Fenvalerate in Tomato by Ultrasound-Assisted Solvent Extraction Combined with Dispersive Liquid–Liquid Microextraction

Meghdad Pirsaeheh1, Toraj Ahmadi-Jouibari2, Nazir Fattahi1* and Mojtaba Shamsipur3

1Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran, 2Clinical Research Development Center, Imam Khomeini Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran, and 3Department of Chemistry, Razi University, Kermanshah, Iran

*Author to whom correspondence should be addressed. Email: nazirfatahi@yahoo.com

Received 8 April 2013; revised 28 July 2013

Ultrasound-assisted solvent extraction (UASE) combined with dispersive liquid–liquid microextraction based on the solidification of floating organic drop (DLLME-SFO) has been developed for extraction and determination of fenvalerate from tomato samples. Fenvalerate was determined by high-performance liquid–liquid chromatography-ultraviolet detector. Effects of parameters such as type and volume of extraction solvent in the UASE stage, sonication time, type and volume of extraction solvent and disperser solvent in the DLLME-SFO stage, salt addition and pH effect on extraction were studied and optimized. Under the optimum conditions, the calibration graph was linear in the range of 5–500 μg kg⁻¹ with a detection limit of 0.6 μg kg⁻¹. The relative standard deviation for five replicate measurements of 100 μg kg⁻¹ of fenvalerate was 6.5%. The relative recovery of fenvalerate in different tomato samples at a spiking level of 10, 20 and 50 μg kg⁻¹ is in the range of 93.5–108%. The obtained results show that UASE–DLLME-SFO is a sensitive, fast and simple method for the determination of fenvalerate in tomato samples.

Introduction

In recent years, pyrethroids have widely been used as a new type of pesticide to control pests in agriculture, households, public health, forestry, horticulture and veterinary medicine because of their selective insecticidal activity, rapid biotransformation and excretion by the mammalian catabolic system and their non-persistence in the environment (1). The synthetic pyrethroids can cause serious health effects, such as paresthesia, headache, dizziness, nausea and skin irritation, to humans (2). Fenvalerate is a member of the pyrethroid family and has been classified as a human carcinogen and most widely used pesticide because of its excellent insecticidal properties (3). For this purpose, fenvalerate has a high range of use in Iran, particularly in Kermanshah province. The European Union regulation has recently set up (established) a maximum residue limit of 0.02 mg kg⁻¹ of fenvalerate in vegetables.

A variety of analytical approaches have been proposed for the trace-level analysis of fenvalerate in different samples such as gas chromatography (GC) (4–6), high-performance liquid chromatography (HPLC) (7, 8), thin-layer chromatography (9, 10), gas–liquid chromatography (11, 12) and enzyme immunoassays (13, 14), among which HPLC and capillary GC being of more practical interest. HPLC is the most common method used for the determination of fenvalerate, because it can be coupled with the variety of detectors. Sample preparation plays an important role in a whole analytical process to concentrate the target analytes and to decrease the interferences from a sample matrix. Several pretreatment methods such as solid-phase extraction (SPE) (15), liquid–liquid extraction (LLE) (16), solid-phase microextraction (SPME) (17), stir bar sorptive extraction (SBSE) (18) and single-drop microextraction (SDME) (19) have been developed for analysis of pyrethroid residues. However, the methods mentioned above had some drawbacks. SPE and LLE are time consuming and expensive, whereas the LLE method is expensive and requires large amount of potentially toxic solvents. SPME is expensive and its fiber is fragile and has a limited lifetime; sample carry-over is also a problem. SBSE is also time consuming and, in most cases, equilibrium could not be attained after a long time. SDME suffers from some drawbacks: fast stirring may break up the organic solvent drop, air bubble formation, time consuming and, in most cases, equilibrium is not attained even after a long time.

In recent years, Assadi and coworkers demonstrated a novel microextraction method called dispersive liquid–liquid microextraction (DLLME) (20). DLLME is based on the ternary component solvent system (aqueous sample, dispersive solvent and extraction solvent). In this method, an appropriate mixture of extraction solvent (organic solvent) and dispersive solvent (water-organic miscible solvent) is rapidly injected into the aqueous sample by a syringe; thereby, a cloudy solution is formed. After centrifugation, the analytes are separated into organic phase (extraction solvent). The advantages of DLLME are the usage of a small volume of organic solvents, ease of operation, rapidity, low cost, high recovery and high enrichment factor (EF). Up to now, DLLME has been successfully applied to the determination of organic and inorganic species in different matrices (21–25).

In conventional DLLME, the density of extraction solvent should be higher than water; the applications of DLLME, in most cases, were limited for water samples and the volume of the sedimented phase, in some cases, was dependent on the surrounding temperature. In addition, high-density extraction solvents, being mostly halogenated, are generally hazardous to laboratory personnel and the environment. Recently, less toxic solvents, such as alcohols, alkanes, etc., with density lighter than water are collected on the upper surface of the sample solution as a microdrop or a thin film after centrifuging. Removal and determination of the collected phase volume are not as simple as that of high-density extraction solvents. Different techniques such as using a capillary tube (26) or specialized extraction vessel (27) were reported for the removal of lighter extraction solvents. However, the complete removal of the collected phase is difficult or impossible in most of the cases. A novel DLLME method based on the SFO (DLLME-SFO) was introduced by Leong and Huang (28). In DLLME-SFO, the
UASE–DLLME−SFO technique. The practical applicability of the method was investigated for the extraction and determination of fenvalerate in tomato samples.

**Calculations of EF, extraction recovery and relative recovery**

EF was defined as the ratio between the concentration of an analyte in the floated phase (∼C_{floated}) and the initial concentration of the analyte (∼C_0) within the sample.

\[
EF = \frac{C_{floated}}{C_0}
\]

The extraction recovery (ER%) was defined as the ratio between the amount of an analyte in the floating phase (∼n_{floated}) and the initial amount of the analyte (∼n_0) within the sample.

\[
ER\% = \frac{n_{floated}}{n_0} \times 100 = \frac{C_{floated} V_{floated}}{C_0 V_{sample}} \times 100
\]

where \( V_{floated} \) and \( V_{sample} \) are the volumes of the floating phase and sample, respectively.

The relative recovery (RR%) was obtained from the following equation:

\[
RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100
\]

where \( C_{found} \), \( C_{real} \) and \( C_{added} \) are the total concentration of an analyte after addition of a known amount of standard in real sample, the original concentration of the analyte in real sample and the concentration of a known amount of standard that was spiked to the real sample, respectively.

**Results**

In the present work, UASE−DLLME−SFO combined with HPLC−UV was used for simultaneous preconcentration and determination of fenvalerate in tomato samples. In this new combination, to reach a high extraction recovery and EF, the UASE and DLLME conditions must be examined and optimized. Some effective parameters such as sonication time, extraction solvent in the UASE stage, extraction and disperser solvent type and their volume in the DLLME stage, salt addition and effect of pH were studied.
Optimization of UASE parameters

Optimization of sonication time

Our attempts were primarily centered on optimizing sonication time under extraction conditions. To investigate the effect of sonication time on extraction recovery, additional experiments were performed using different sonication times, from 5 to 40 min. An ultrasonic device with fixed power of 350 W was used. When the sonication time increased from 5 to 30 min, the recovery of fenvalerate was increased due to mass transfer of analyte from cellular material to acetone by diffusion and osmosis (33). However, the extraction efficiency had no noticeable enhancement when the sonication time increasing from 30 to 40 min. Thus, 30 min was selected as the optimum sonication time.

Selection of extractant solvents in the UASE stage

When combining UASE with DLLME, the extracting solvent in the UASE stage must also play the role of the disperser solvent at the DLLME stage. For this purpose, acetone, acetonitrile and methanol were selected as the extracting solvents in the UASE stage (33). The effect of different solvents on the extraction recovery of fenvalerate is shown in Figure 1. As shown Figure 1, the best extraction recovery was obtained when using acetone as the extraction solvent in the UASE stage. Therefore, acetone was selected as the extraction solvent in further experiments.

Optimization of DLLME parameters

Type of the extraction solvent and ratio of solvent to material

The extracting solvent in the DLLME stage must have low volatility, low water solubility, high solubility in dispersive solvent, capable of forming cloudy solution in water in the presence of dispersive solvent, a melting point close to room temperature (in the range of 10–30°C), no interference with the analytical techniques used for the determination of an analyte and density lower than water. Several extracting solvents, including 1-decanol, 1-dodecanol and 1-undecanol, were tested to determine the best extraction solvent in the DLLME stage. Some characteristics of the extraction solvents and disperser solvents are shown in Table 1. The recovery of fenvalerate using different extraction solvents is shown in Figure 2. According to the results shown in Figure 2, recovery of fenvalerate in the presence of 1-undecanol is higher than the other tested solvents. Therefore, 1-undecanol was chosen for further experiments.

The volume of 1-undecanol was also an important factor for the extraction of fenvalerate. To investigate the effect of extraction solvent volume on recovery, additional experiments were performed by using different volumes of 1-undecanol (30.0, 40.0, 50.0, 60.0 and 70.0 µL). The volume of the floating phase increases with the increase in extraction solvent volume; however, the analyte becomes diluted as a result of the increase in the volume of the floating phase and EF decreases (Figure 3). Although the use of lower volumes of extraction solvent might lead to higher EF, it was not easy to handle extracts with volumes <30.0 µL. Therefore, 30.0 µL was selected as the extraction solvent volume.

Types of the disperser solvents and their volume

When combining UASE with DLLME-SFO, the extraction solvent used in the UASE stage must also play the role of the disperser solvent at the DLLME stage. Therefore, acetone, acetonitrile and methanol were selected for this purpose. According to Selection of extractant solvents in the UASE stage section, acetone was selected as the disperser solvent.

Table I. Properties of Extraction Solvents and Disperser Solvents for the DLLME-SFO Method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g mL⁻¹)</th>
<th>Solubility in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction solvent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Decanol</td>
<td>6–8</td>
<td>233</td>
<td>0.83</td>
<td>Insoluble</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>13–15</td>
<td>243</td>
<td>0.83</td>
<td>Insoluble</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>22–24</td>
<td>259</td>
<td>0.83</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Disperser solvent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>−95</td>
<td>56–57</td>
<td>0.79</td>
<td>Soluble</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>−46</td>
<td>81–82</td>
<td>0.78</td>
<td>Soluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>−97.6</td>
<td>64.7</td>
<td>0.79</td>
<td>Soluble</td>
</tr>
</tbody>
</table>

Figure 1. Effect of type of extraction solvent in the UASE stage on the recovery of fenvalerate from tomato samples. Extraction conditions: sonication time, 30 min; volume of extraction solvent in the UASE step, 5 mL; sample amount, 1.0 g; extraction solvent in the DLLME step and its volume, 1-undecanol, 30 µL; volume of disperser solvent (acetone), 1000 µL; sedimented phase volume, 25 ± 1 µL; room temperature; concentration of fenvalerate 100.0 µg kg⁻¹; all experiments were performed in triplicates (n = 3).

Figure 2. Effect of type of extraction solvent in the DLLME stage on the recovery of fenvalerate from tomato samples. Extraction conditions: as in Figure 1 except for the type of extraction solvent in the UASE stage, acetone.
EF of fenvalerate from tomato samples. Extraction conditions: as in Figure 1 except for the type of extraction solvent in the UASE stage, acetone.

Figure 3. Effect of volume of extraction solvent in DLLME stage (1-undecanol) on the EF of fenvalerate from tomato samples. Extraction conditions: as in Figure 1 except for the type of extraction solvent in the UASE stage, acetone.

Figure 4. Effect of volume of disperser solvent (acetone) on the recovery of fenvalerate from tomato samples. Extraction conditions: as in Figure 1 except for the type of extraction solvent in the UASE step, acetone.

For obtaining optimized volume of acetone, experiment was repeated using different volumes of acetone (250, 500, 1000, 1500 and 2000 μL), containing different volumes of 1-undecanol to obtain the constant volume of the floated phase (25 ± 1 μL) in all experiments. In agreement with the respective results (Figure 4), the extraction efficiency initially increases, afterwards it reduces as the acetone volume increases. This observation could be attributed to the fact that at lower acetone volume, the cloudy suspension of the 1-undecanol droplets was not formed well, resulting in a decrease in the extraction recovery. At higher acetone volume, the solubility of fenvalerate in water increased and the extraction efficiency reduced. Therefore, 1000 μL of acetone was selected as the optimum volume for the disperser solvent.

Effect of extraction time and ionic strength
In DLLME-SFO, extraction time is defined as interval between injecting the mixture of disperser solvent and extraction solvent, before starting to centrifuge. The extraction time is an important factor that may affect the analytes’ extraction efficiency from aqueous phase into the organic phase. Thus, the variation in extraction efficiency of fenvalerate as a function of extraction time was studied in the range of 0–20 min. The resulting data show that the extraction time has no significant effect on the extraction efficiency for the target analyte. It was found that, after the formation of the cloudy solution, the contact area between the extraction solvent and the aqueous phase was considerably large, delineating why the extraction equilibrium could be established very fast.

For investigating the influence of the ionic strength on the DLLME-SFO performance, several experiments were performed by adding varying the concentration of NaCl, from 0 to 5% (w/v). The results demonstrated an improvement in the formation of floated drop and extraction efficiency for fenvalerate up to 1% (w/v). Increasing the concentration of NaCl >1% (w/v) causes a small decrease in the efficiency. Therefore, 1% NaCl (w/v) was used in further experiments.

Figures of merit of the proposed method
The analytical figures of merits of the proposed method were investigated under the optimized experimental conditions. Calibration curves were obtained under the optimized conditions with a linear dynamic range of 5–500 μg kg⁻¹ and a correlation coefficient (r²) of 0.9986. The extraction recovery of the method was 55.2%, at the concentration level of 100 μg kg⁻¹ of fenvalerate. The RSD (n = 5) at the concentration level of 100 μg kg⁻¹ was 6.5%. The limit of detection (LOD) based on the signal-to-noise ratio (S/N) of 3 was 0.6 μg kg⁻¹.

Determination of fenvalerate in field samples
To validate the proposed method, field tomato samples obtained from Miandarband and Ravansar regions (province of Kermanshah, Iran), which was treated with fenvalerate, were analyzed following the UASE–DLLME-SFO procedure in triplicates. The results showed that the analyzed samples were free of fenvalerate contamination. These samples were spiked with the standard of fenvalerate at different concentration levels to assess matrix effects. Figure 5 shows the obtained chromatograms of tomato sample (A) and spiked tomato sample at the concentration level of 50 μg kg⁻¹ for fenvalerate (B). The results of relative recovery of tomato samples are shown in Table II. The data in Table II show that the relative recoveries of fenvalerate were in the range of 93.5–108%, demonstrating that the tomato sample matrices had little effect on the UASE–DLLME-SFO method.

Comparison of UASE–DLLME-SFO with other extraction methods
Characteristics of the proposed method have also been compared with other methods that were used for the preconcentration and determination of fenvalerate in different samples. Table III compares LOD, linear range (LR) and repeatability. UASE–DLLME-SFO has the shortest extraction time and lowest LOD. The LR of the UASE–DLLME-SFO method is better than other methods and the RSD of the proposed method is acceptable. Comparison of the other procedures with UASE–DLLME-SFO indicated that UASE–DLLME-SFO demonstrate lower LOD, very short extraction time and ease of operation. Also, in comparison with conventional DLLME, UASE–DLLME-SFO used lower toxicity solvents and shows slightly higher extraction recovery of fenvalerate in different samples.
**Discussion**

This study explored the applicability of UASE–DLLME-SFO to determine the concentration of fenvalerate in tomato samples. The effects of several variables, including sonication time, extraction solvent in UASE stage, extraction and disperser solvent types and their volume in the DLLME stage, salt addition and effect of pH, were investigated and optimized to achieve high sensitivity of the proposed method. The UASE used for the extraction of compounds from semisolid and solid matrices was combined with DLLME (UASE–DLLME) as a sample preparation method for semisolid and solid samples. The UASE–DLLME-SFO method requires extraction solvents with lower toxicity instead of highly toxic solvents by conventional DLLME, and it provides high extraction recovery within a short time.

Table II. Relative Recoveries and Standard Deviations of Fenvalerate from Spiked Tomato Samples

<table>
<thead>
<tr>
<th>Field no.</th>
<th>Added (µg kg⁻¹)</th>
<th>Found mean (n = 3) ± SD (µg kg⁻¹)</th>
<th>Relative recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10.8 ± 0.8</td>
<td>108.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.4 ± 2.7</td>
<td>98.0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>18.7 ± 1.5</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>41.3 ± 3.2</td>
<td>103.2</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>53.0 ± 3.7</td>
<td>106.0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>78.6 ± 5.8</td>
<td>98.2</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>19.3 ± 2.2</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>56.1 ± 4.6</td>
<td>93.5</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>47.6 ± 3.8</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>103.0 ± 7.5</td>
<td>103.0</td>
</tr>
</tbody>
</table>

SD, standard deviation (n = 3).

Under the optimized UASE–DLLME-SFO conditions, as shown in Figure 5, the selected chromatographic conditions resulted in good chromatographic resolution. The RSD (n = 5) at the concentration level of 100 µg kg⁻¹ was 6.5%, and the LOD based on the S/N of 3 was 0.6 µg kg⁻¹. Calibration curves were obtained under the optimized conditions with a linear dynamic range of 5–500 µg kg⁻¹ and a correlation coefficient (r²) of 0.9986. The extraction recovery of the method was 55.2%, at the concentration level of 100 µg kg⁻¹ of fenvalerate. The results given in Table II indicated that fenvalerate was not found in all of the tomato samples. These samples were spiked with the standard of fenvalerate at different concentration levels to assess matrix effects. The relative recovery values for the spiked samples were in the range of 93.5–108% and standard deviation (SD) was not higher than 7.5.

**Conclusions**

This article describes an UASE–DLLME-SFO method combined with HPLC–UV that is applied to the analysis of fenvalerate in tomato samples. The results of this study demonstrate that the proposed method provides acceptable recovery and repeatability values for fenvalerate from tomato samples. The proposed method is simple, effective and environmentally friendly for the separation and preconcentration of fenvalerate in vegetables. Additionally, it is a low-cost technique, because it uses small amounts of solvent and sample solution throughout the experiment. The method could be extended to other analytes and other types of fruit and vegetable samples.

**Funding**

The authors thank the Deputy of Research and Technology, Kermanshah University of Medical Sciences (Kermanshah, Iran) for financial support.

**References**

using liquid phase microextraction coupled in-syringe derivatization followed by gas chromatography/electron capture detection; Analytical and Bioanalytical Chemistry; (2011); 401: 927–937.


9. Patil, V.B., Sevalkar, M.T., Paddalkar, S.V.; Thin-layer chromatographic detection of pyrethroid insecticides containing a nitrile group; Analyst; (1992); 117: 75–76.


15. Woudneh, M.B., Oros, D.R.; Quantitative determination of pyrethroids, pyrethrins, and piperonyl butoxide in surface water by high-resolution gas chromatography/high-resolution mass spectrometry; Journal of Agricultural and Food Chemistry; (2006); 54: 6957–6962.


18. Hoeck, E.V., David, F., Sandra, P.; Stir bar sorptive extraction for the determination of pyrethroids in water samples: a comparison between thermal desorption in a dedicated thermal desorber, in a split/splittless inlet and by liquid desorption; Journal of Chromatography A; (2007); 1157: 1–9.


25. Liang, P., Zhang, L., Zhao, E.; Displacement-dispersive liquid–liquid microextraction coupled with graphite furnace atomic absorption spectrometry for the selective determination of trace silver in environmental and geological samples; Talanta; (2010); 82: 993–996.

26. Farajzadeh, M.A., Djozan, D.J., Bakhtiyari, R.E.; Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid–liquid microextraction and its application in the determination of parabens in different samples by gas chromatography–Flame ionization detection; Talanta; (2010); 81: 1360–1367.

27. Biparva, P., Ehsani, M., Hadjimohammadi, M.R.; Dispersive liquid–liquid microextraction using extraction solvents lighter than water combined with high performance liquid chromatography for determination of synthetic antioxidants in fruit juice samples; Journal of Food Composition and Analysis; (2012); 27: 87–94.


29. Chang, C.C., Huang, S.D.; Determination of the steroid hormone levels in water samples by dispersive liquid–liquid microextraction with solidification of a floating organic drop followed by high-performance liquid chromatography; Analytica Chimica Acta; (2010); 662: 39–43.


32. Mirzaei, M., Behzadi, M., Mahmoud Abadi, N., Beizaei, A.; Simultaneous separation/preconcentration of ultra trace heavy metals in industrial wastewaters by dispersive liquid–liquid microextraction based on solidification of floating organic drop prior to determination by graphite furnace atomic absorption spectrometry; Journal of Hazardous Materials; (2011); 186: 1739–1743.
