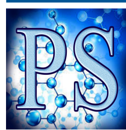
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Research Article

Control of Organophosphorus Pesticides Residues in Honey Samples Using a Miniaturized Tandem Preconcentration Technique Coupled with High Performance Liquid Chromatography

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Abstract

Background: In the present work, a miniaturized sample preparation method based on combination of dispersive solid phase extraction and temperature–induced homogenous liquid–liquid microextraction has been proposed for the extraction and preconcentration of some organophosphorus pesticides (parathion–methyl, triazophos, parathion, diazinon, and phoxim) from honey samples prior to their analysis by high performance liquid chromatography–ultraviolet detection.

Methods: In this method, initially the analytes were adsorbed onto a sorbent (C_{18}) and then desorbed by the use of cyclohexyl amine as an eluent. In the next step, the eluent was mixed with water thermostated at 0 °C to obtain a homogenous solution. By increasing the temperature, the solubility of cyclohexyl amine in water was decreased and led to formation of dispersed fine droplets in the whole of solution. These droplets go up through the solution and collected on top of the solution. Finally, an aliquot of the organic phase was sucked in a microsyringe and injected into the separation system for analysis.

Results: Under the optimum experimental conditions, limits of detection and quantification were calculated to be in the ranges of 0.90–1.75 and 3.0–5.8 ng g^{-1} in honey samples, respectively. Enrichment factors and extraction recoveries were in the ranges of 148–183 and 59–73%, respectively. The relative standard deviations varied from 2–4% and 4–5% for intra– ($n = 6$) and inter–day ($n = 4$) precisions, respectively.

Conclusion: The suggested approach was satisfactorily utilized to the analysis of 21 honey samples. The proposed miniaturized tandem sample pretreatment method enhanced the sensitivity of the instrumental analysis.

Introduction

During the last decades, pesticides have been used all over the world to control pests and obtain the high quality of agriculture products. The organophosphorus pesticides have been utilized extensively due to their low prices and relatively short environmental persistence. When they are used improperly in the agricultural activities, they may reach to humans and animals' food/feed and ultimately may cause detrimental effects on human health. OPPs can be toxic when they are absorbed by human organisms because of acetyl–cholinesterase deactivation.[1,2]

To minimize the risk for human health, food authorities tried to establish regulations such as maximum residue level (MRL) for pesticides including OPPs. To ensure that MRL for certain pesticides are controlled, their residues determination in food is essentially required. Honey is a food product consumed by human all over the world as the rich source of vitamins, sugars, carbohydrates, amino acids, and minerals such as calcium and magnesium. Therefore, it is essential to provide an efficient method for quantifying harmful pollutants such as pesticides residues in honey samples to warrant consumers' safety.[3,4]

Several chromatographic methods such as high–performance liquid chromatography (HPLC),[5–7] liquid

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chromatography–tandem mass spectrometry (LC–MS–MS),[8] and gas chromatography–mass spectrometry (GC–MS)[1,9,10] have been used for OPPs analysis in different samples. Although these methods are sensitive and selective, another stage named as sample preparation is needed before instrumental analysis because of trace levels of pesticides present in the real samples.[11] Recently, various miniaturized sample preparation techniques called microextraction methods were used in extraction of various analytes and chemicals from different samples.[12–15] Liquid–liquid extraction and solid phase extraction (SPE) are well–known extraction procedures. But these methods are not environmentally friendly because of the consumption of large volumes of organic solvents. The applicability of SPE and solid phase microextraction has always been restricted to a limited number of commercially available cartridges and fibers that are expensive and have limited life time.[16,17] Also, both of them are time–consuming. To overcome these deficiencies, new methods have been developed in which the sorbents can be dispersed in the sample solution. These methods were termed as dispersive solid phase extraction (DSPE) or dispersive solid phase microextraction.[16,18] In these methods, the sorbent is directly dispersed into the sample. This phenomenon improves the contact interface between analytes and sorbent which leads to increase extraction efficiency.[18] After centrifugation, the sorbent is eluted with a solvent to desorb the retained analytes. Mostly sorbents like primary secondary amine (PSA), C_8 (octyl), and C_{18} (octadecyl) are used in these methods.[16,19] The main disadvantage of these methods is low enrichment factor (EF) because of the consumption of high volume of an eluent leading to the high detection limits. To solve this deficiency, it is necessary to vaporize the solvent which has its own problems. The application of microextraction methods such as dispersive liquid–liquid microextraction (DLLME) and homogeneous liquid–liquid microextraction (HLLME) can be the other choice. In HLLME, analytes existing in a homogeneous solution are extracted into a water–miscible organic solvent (at µL level) by changing the temperature, pH or ionic strength.[20,21] In HLLME, the extraction speed is high because of the absence of obstacles between the aqueous phase and the organic phase during the extraction procedure. Unlike DLLME, HLLME does not need any disperser solvent. The disperser solvent increases the solubility of the analytes into the aqueous phase and consequently the extraction efficiency is decreased.[22]

The purpose of current study was to propose an efficient extraction method with combining DSPE and temperature–induced homogeneous liquid–liquid microextraction (TI–HLLME) for the extraction of five OPPs from honey samples prior to their determination by high performance liquid chromatography–ultraviolet detector (HPLC–UV). As far as we know, up to now there was no report on the application of the proposed approach for the quantification of target pesticides in honey samples. Also, for the first time, µL–level of cyclohexyl amine was utilized as an eluent and extractant simultaneously, that cause to reduce the use of conventional halogenated solvents. This can be a great merit in the era of green chemistry. Unfortunately, DSPE has relatively low EFs in most cases. Therefore to obtain high EFs and low limits of detection (LODs) it was combined with TI–HLLME. This combination also solved the problem associated with TI–HLLME in its application in the analysis of samples having complex matrices.

Materials and Methods

Reagents and solutions

Five OPPs including triazophos, diazinon, phoxim, parathion, and parathion–methyl were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Methanol, HPLC–grade water, sodium chloride, hydrochloric acid (37%, *w/w*), and sodium hydroxide were supplied from Merck (Darmstadt, Germany). Cyclohexyl amine and 3–picoline were purchased from Sigma–Aldrich (Louis, USA). The consumed sorbents (C_{18}, C_8, and PSA) were purchased from Merck. A mixture stock standard solution (100 mg L^{-1}) of pesticides was prepared in methanol. The working standard solutions were daily prepared by appropriate dilution of the stock solution with HPLC–grade water.

Real samples

Twenty–one honey samples were supplied from vendors in the West and East Azerbaijan Provinces–Iran. Another sample was obtained from beehives placed in virgin mountainous lands (Kaleybar, East Azerbaijan Province, Iran) that was completely far away from the agricultural zones and used as a blank sample in the procedure.

Instrumentation

A Hewlett–Packard 1090–II HPLC system (Palo Alto, CA, USA) equipped with an UV detector operating at 245 nm was utilized for quantitative analysis of the analytes. A Centurysil C_{18} column from Dalian Jiangshen Company (Dalian, China) (25 cm × 4.6 mm i.d., 5 µm particle size) was utilized for separation. The mobile phase was a mixture of methanol–water (75:25, v/v) at a flow rate of 1.0 mL min^{-1} using an isocratic elution. The HPLC system was controlled using ChemStation software. A Universal 320 R centrifuge (Hettich, Kirchlengern, Germany) and a Metrohm pH meter (model 654, Herisau, Switzerland) were utilized for accelerating phase separation and pH adjustment, respectively. Also, a vortex mixer (Velp Scientific, Italy) was utilized for vortexing the sample solution.

Extraction procedure

DSPE

As schematically shown in Figure 1, 1 g of pesticide–free honey sample was transferred into a 10–mL glass test tube and brought to 5 mL by HPLC–grade water. The solution was spiked with the analytes (200 ng g^{-1} of each pesticide) and 100 mg of C_{18} was added to it. Then to ensure the complete dispersion of C_{18}, mixture was vortexed for 3 min. The supernatant was decanted after centrifuging the

mixture for 4 min at 5000 rpm. For desorption the analytes, the adsorbent was eluted with 175 µL cyclohexyl amine under sonication for 3 min. The obtained elution solvent was utilized as an extractant in the following TI–HLLME procedure.

TI–HLLME

In this step, the collected eluent obtained from the DSPE step was added into 5 mL HPLC–grade water placed into a 10–mL test tube which held into an ice bath for 2 min. A homogeneous solution was formed (cyclohexyl amine was dissolved completely) by manual shaking and the analytes were dissolved in the whole of HPLC–grade water (analytes were not present in cyclohexyl amine anymore). In the next step, the tube was transferred into a water bath thermostated at 60 °C for 3 min. By doing so, the homogenous solution was broken (the solubility of extractant in the aqueous phase was decreased at 60 °C) and the analytes were extracted into the tiny droplets of produced cyclohexyl amine droplets. It should be mentioned that cyclohexyl amine is miscible with HPLC–grade water at 0 °C while only 160 µL of it is dissolved in 5 mL HPLC–grade water. After centrifuging for 4 min at 5000 rpm, the organic phase (20 ± 1 µL) containing the extracted analytes was collected on the top of the aqueous phase. In the following, 10 µL of the collected phase was isolated and injected into the HPLC–UV by an auto injector.

Calculation of EF and extraction recovery (ER)

EF is considered as the analyte concentration ratio in the collected phase (C_c) to its initial concentration (C_0) in the sample.

$$EF = C_c / C_0 \qquad \text{Eq. (1)}$$

ER refers to the percentage of the total analyte amount (n_0) which is extracted into the collected phase (n_c).

$$ER\% = \frac{n_c}{n_0} \times 100 = \frac{C_c \times V_c}{C_0 \times V_{aq}} \times 100 = EF \times \frac{V_c}{V_{aq}} \times 100 \qquad \text{Eq. (2)}$$

Where V_c and V_{aq} are volumes of collected organic phase and aqueous solution, respectively.

Results and discussion

Optimization of DSPE step

Optimization of type and amount of sorbent

The sorbent type can influence analytical performance of DSPE. In this work, sorbent materials used for DSPE were C_{18}, PSA, and C_8. In order to select the sorbent, 150 mg of each sorbent was added into the diluted honey sample spiked with analytes (200 ng g^{-1} of each analyte). According to the results in Figure 2, C_{18} is the best sorbent among the evaluated sorbents considering extraction efficiencies. This phenomenon can be related to the high hydrophobicity of C_{18} in comparison with other used sorbents. Therefore, C_{18} was chosen for the further studies.

The amount of the sorbent is a critical factor in DSPE method since it can affect the amount of the analytes adsorbed on the surface of the sorbent and subsequently the ERs of analytes. So, it was evaluated in the range of 25–200 mg. Considering the outcomes in Figure 3, ERs enhanced by increasing C_{18} amount from 25 to 100 mg and

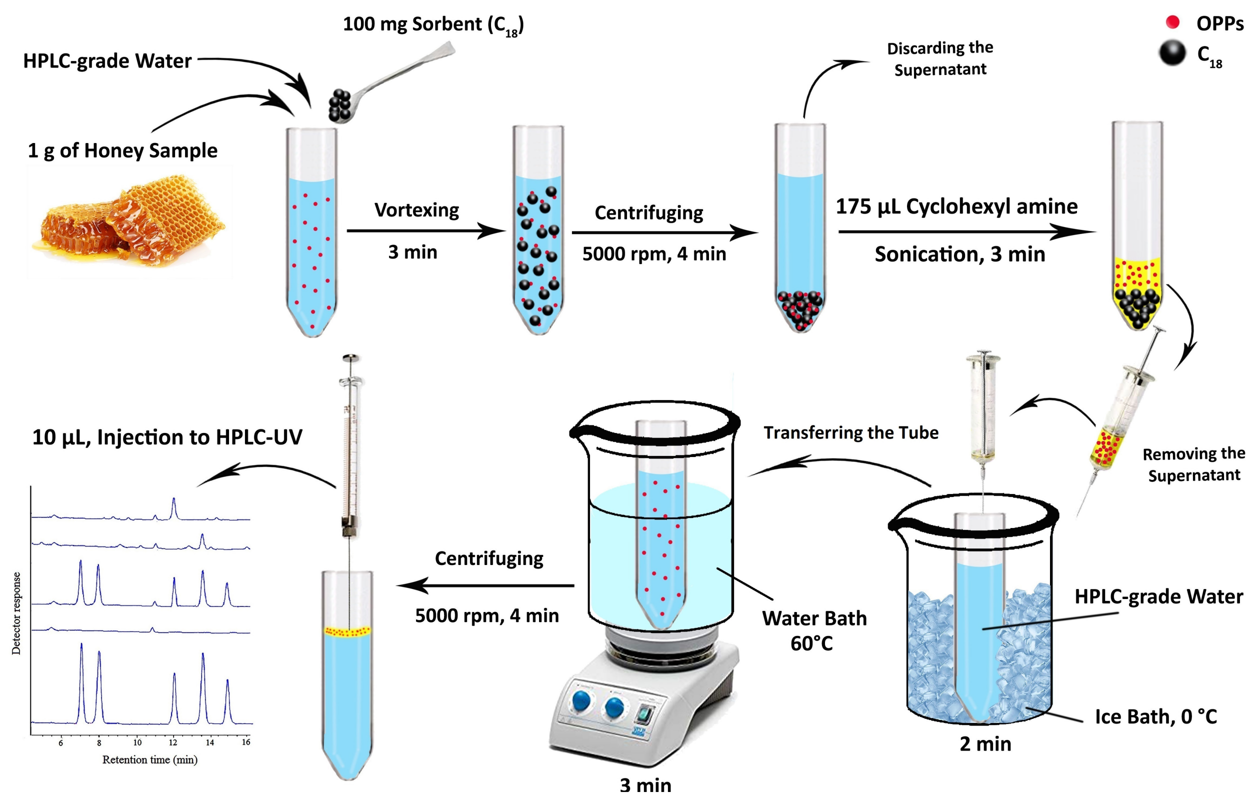

Figure 1. DSPE–TI–HLLME–HPLC–UV procedure.

afterward reached to a constant amount with additional sorbent. So, 100 mg was chosen as an optimum.

Optimization of vortex time

In presented work, vortex agitation was utilized for the dispersion of sorbent which could enhance the contact interface between analytes and sorbent and can led to improve extraction efficiency. To determine the ideal time, the sample was vortexed for 1–5 min. The obtained results showed that the ERs increased with enhancing the vortex time till 3.0 min and then remained constant. Therefore, 3.0 min was opted for the accomplishment of the further experiments.

Salt addition

In DSPE, addition of salt can influence the ionic strength of media and subsequently the performance of the proposed method. Thus, the effect of NaCl concentration was investigated in the range of 0–10% (*w/v*). According to the obtained outcomes, addition of salt had a negative effect on the ERs of analytes and by increasing NaCl concentration, the ERs slightly decreased. The decrease in the ERs of analytes can be related to the occupation of the active sites on the surface of the used sorbent by salt. So, the subsequent studies were performed without salt.

Effect of sample solution pH

Sample solution pH has a crucial effect on the ERs of the analytes as it determines the analytes forms (ionic or neutral forms). The pH of sample solution was investigated from 2 to 12 using suitable volumes of 1 M HCl or NaOH solutions. Considering the results in Figure 4, ERs of analytes were

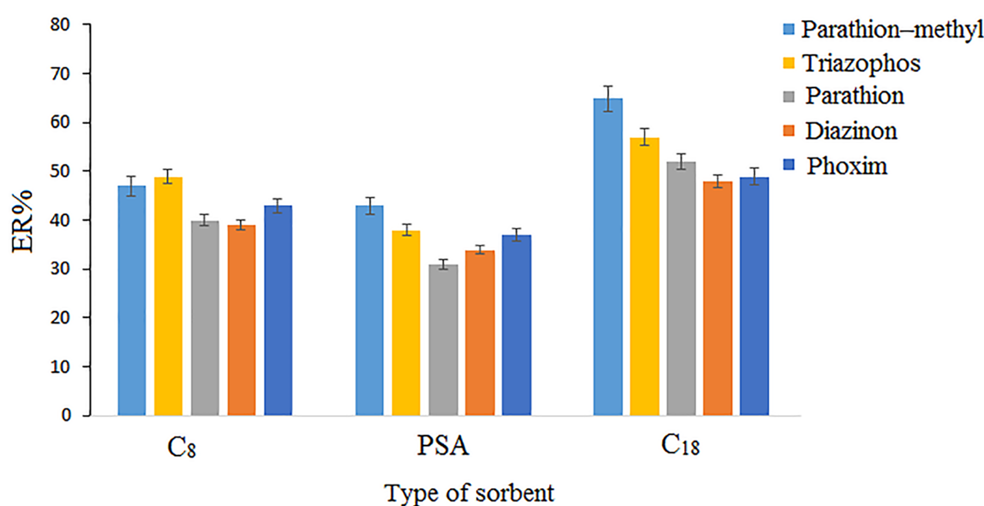

Figure 2. Selection of sorbent type. Extraction conditions: DSPE: aqueous sample, 5 mL diluted pesticides–free honey sample solution spiked with analytes (200 ng g^{-1}, each pesticide); sorbent amount, 150 mg; vortex time, 5 min; desorption/extraction solvent (volume), cyclohexyl amine (175 µL); and desorption time, 5 min. TI–HLLME: aqueous phase, 5 mL HPLC–grade water placed into the ice bath for 2 min; heating temperature, 70 °C; heating time, 2 min; centrifugation time, 5 min; and centrifugation rate, 5000 rpm. The error bars indicate minimum and maximum of three determinations.

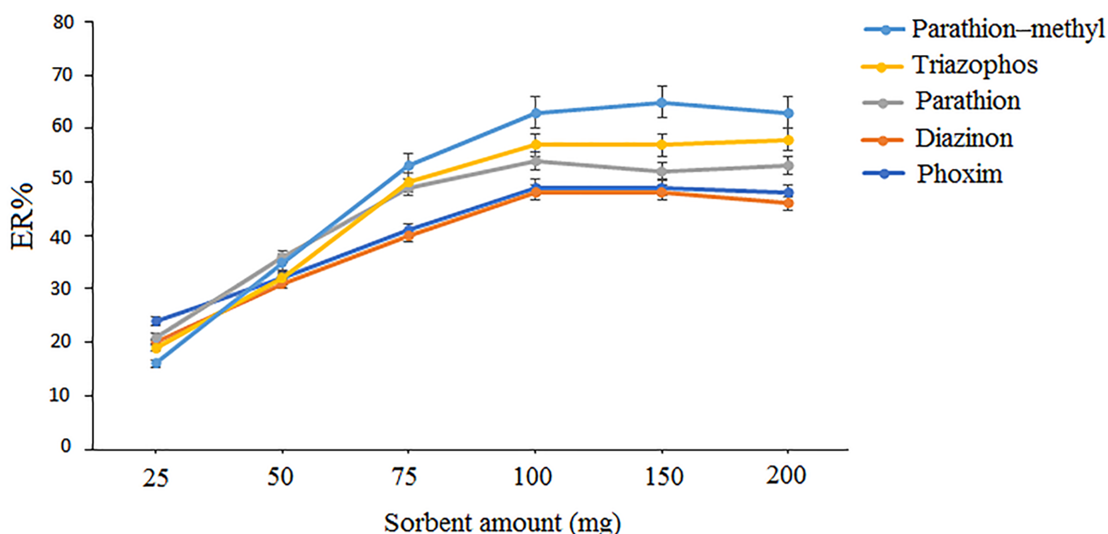

Figure 3. Optimization of sorbent amount. Extraction conditions: are the same as used in Figure 2, except C_{18} was used as the sorbent.

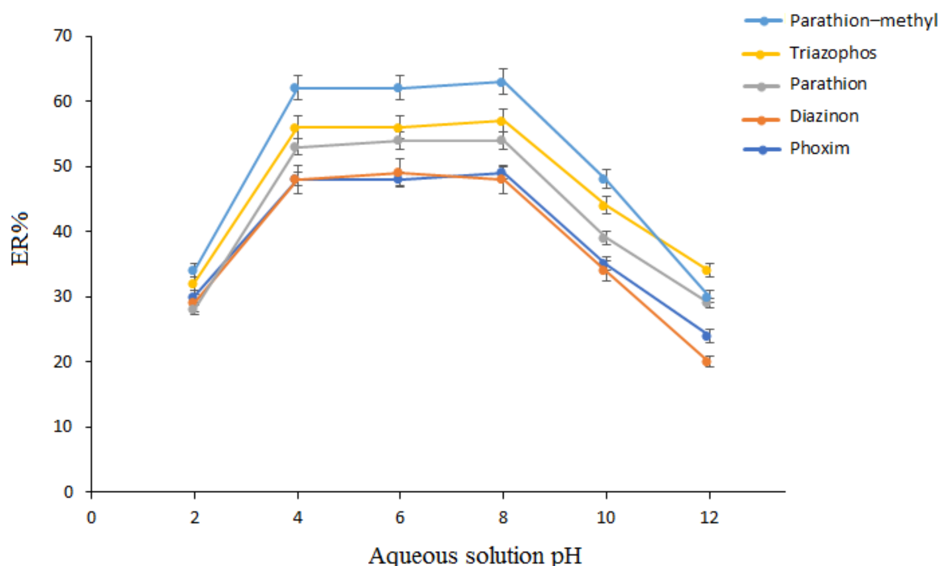

Figure 4. Study of aqueous solution pH. Extraction conditions: are the same as used in Figure 3, except 100 mg of C_{18} and 3 min were used as the amount of sorbent and vortex time, respectively.

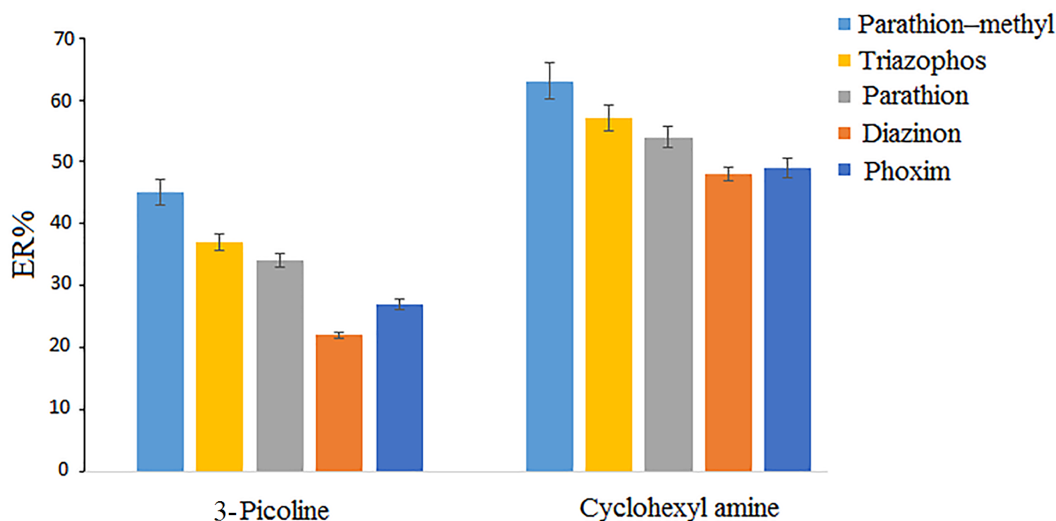

Figure 5. Selection of desorption/extraction solvent. Extraction conditions: are the same as used in Figure 4, except experiments were done without adjustment of aqueous solution pH.

at their maximum value when the pH of aqueous solution was kept in the range of 4 to 8 and decreased remarkably in the strong acidic or alkaline sample solution. There is no need for pH adjustment in this study, since the pH of elevated samples were between 4 and 8.

Selection of type and volume of desorption/extraction solvent

In proposed method, desorption solvent used in DSPE also acts as an extractant in the following TI–HLLME procedure. An appropriate desorption/extraction solvent should meet several criteria such as ability to form a two–phase system by changing temperature, good chromatographic behavior, low density compared to water, and high extraction capability of analytes. Considering these criteria, 3–picoline and cyclohexyl amine were tested in this step. To reach the same collected phase volume (20 ± 1 µL), various volumes of the above–mentioned solvents (150 µL of 3–picoline and 175 of cyclohexyl amine) were used due to their different solubility in the aqueous solution. As it can be seen in Figure 5, cyclohexyl amine gives higher ERs for the analytes compared to 3–picoline. Therefore, cyclohexyl amine was selected for the accomplishment of the next studies.

To evaluate the influence of desorption/extraction solvent volume on the ERs of analytes, various volumes (160, 170, 175, 185, 200, 225, and 250 µL) of cyclohexyl amine were used. According to the results the ERs increased up to 175 µL and after that remain constant at high volumes of the extraction solvent. The volume of the collected phase after performing the proposed method increased from 8 to 56 µL with increasing the volume of extractant from 160 to 250 µL. Hence, 175 µL cyclohexyl amine was opted as an optimum.

Optimization of desorption time

This factor also can influence the ERs of analytes in the proposed method. In this study, desorption time was evaluated by using sonication from 0 to 5.0 min. According to the results, sonication for 3.0 min was enough for complete desorption of the analytes. Thus, 3.0 min was chosen for the accomplishment of the next experiments.

Optimization of TI–HLLME procedure

In this step, heating temperature and time, salt addition, and centrifuging speed and time can influence analytical signals. This stage was performed using various heating temperature (30, 40, 50, 60, 70, and 80 °C) and time (1, 2, 3, 4, 5, 7, and 10 min), percent of NaCl (0, 2.5, 5.0, 7.5, and 10%, *w/v*), and centrifuging speed (3000, 4000, 5000, 6000, and 7000 rpm) and time (3, 4, 5, 6, and 7 min). The optimum conditions were obtained in the absence of salt, 60 °C and 2 min as the heating temperature and time, and 4 min and 5000 rpm as centrifuging time and speed, respectively.

Quantitative aspects

Analytical characteristics of the suggested approach were investigated under the optimized conditions by evaluating LOD, limit of quantification (LOQ), linear range (LR), relative standard deviation (RSD), correlation coefficient (r^2), EF, and ER. Table 1 lists the analytical characteristics of the approach. The obtained outcomes demonstrate a good linearity for analytes with r^2 ≥0.996. The LOD and LOQ values were investigated on the basis of signal–to–noise ratios (S/N) of 3 and 10, respectively. The LODs ranged from 0.18 to 0.35 ng mL^{-1} and 0.90 to 1.75 ng g^{-1} in solution and honey, respectively. The LOQs ranged from 0.60 to 1.16 ng mL^{-1} and 3.00 to 5.80 ng g^{-1} in solution and honey, respectively. By evaluating the 25 ng g^{-1} standard solutions with respect to each analyte, the repeatability, showed as RSD%, was computed. The obtained RSDs% were in the ranges of 2–4% and 4–5% for intra– (n=6) and inter–day (n=4) precisions, respectively. The EFs and ERs for analytes were in the range of 148–183 and 59–73%, respectively.

Real samples analysis

To demonstrate the usability of suggested approach, it was utilized to analysis the target pesticides in twenty–one various honey samples. Figure 6 shows typical HPLC–UV chromatograms of standard solution (10 mg L^{-1} of each pesticide in methanol), analyte–free honey sample, spiked honey sample (150 ng g^{-1} of each pesticide), and two un–spiked honey samples (samples 6 and 14). According to the chromatograms parathion and diazinon were detected in samples 6 and 14, respectively. The contents of the analytes were shown in Table 2. The other honey samples

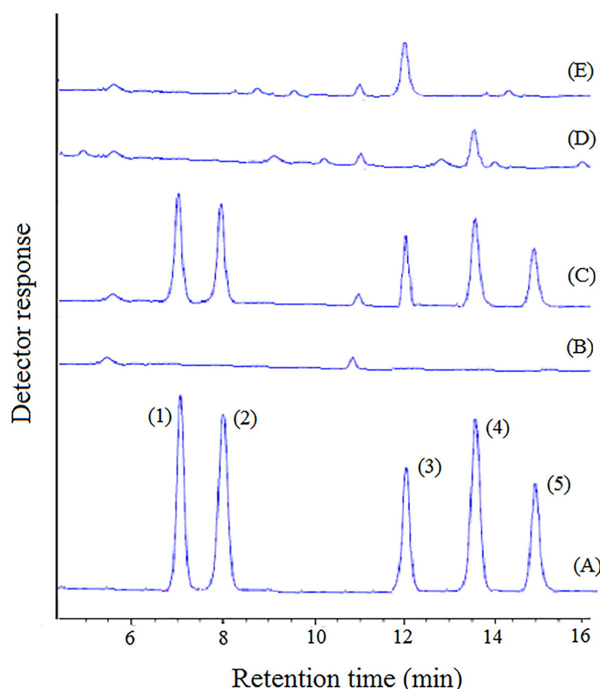

Figure 6. Typical HPLC–UV chromatograms of: (A) standard solution of the selected analytes (10 mg L^{-1} of each analyte), (B) analyte–free honey sample, (C) honey sample spiked with 150 ng g^{-1} of each pesticide, (D) un–spiked honey sample 14, and (E) un–spiked honey sample 6. In chromatogram (A) direct injection without preconcentration was used. Detection wavelength was 245 nm. Peaks identification: 1) parathion–methyl, 2) triazophos, 3) parathion, 4) diazinon, and 5) phoxim.

Table 1. Quantitative features of the developed method for the selected organophosphorus pesticides.

Analyte	LOD [a)]		LOQ [b)]		LR [c)]		r^2 [d)]	RSD% [e)]		EF ± SD [f)]	ER ± SD [g)]
	In solution (ng mL^{-1})	In honey (ng g^{-1})	In solution (ng mL^{-1})	In honey (ng g^{-1})	In solution (ng mL^{-1})	In honey (ng g^{-1})		Intra –day	Inter –day		
Parathion –methyl	0.18	0.90	0.60	3.0	0.60–1000	3.0–5000	0.998	4	4	183 ± 13	73 ± 5
Triazophos	0.26	1.30	0.86	4.30	0.86–1000	4.30–5000	0.997	3	5	173 ± 5	69 ± 2
Parathion	0.33	1.65	1.09	5.45	1.09–1000	5.45–5000	0.998	3	4	168 ± 8	67 ± 3
Diazinon	0.31	1.55	1.03	5.15	1.03–1000	5.15–5000	0.997	2	5	148 ± 8	59 ± 3
Phoxim	0.35	1.75	1.16	5.80	1.16–1000	5.80–5000	0.996	3	4	148 ± 10	59 ± 4

a)Limit of detection (S/N=3).
b)Limit of quantification (S/N=10).
c)Linear range.
d)Determination coefficient.
e)Relative standard deviation for intra– (*n*=6) and for inter–day (*n*=4) precisions at a concentration of 25 ng g^{-1} of each analyte.
f)Enrichment factor ± standard deviation (*n*=3).
g)Extraction recovery ± standard deviation (*n*=3).

were free of the studied pesticides. It should be mentioned that all of the honey samples were analyzed in triplicate and the detected concentrations along with their standard deviations, were reported. The added–found method was utilized to evaluate matrix effect in honey samples. To do this, the honey samples and HPLC–grade water were spiked at two concentrations (30 and 150 ng g^{-1} of each pesticide) and introduced to suggested approach. The obtained peak area for each analyte in the honey samples was divided to that obtained from HPLC–grade water, and the obtained ratio was multiplied by 100. The obtained data, expressed as relative recoveries, are shown in Table 3. The obtained relative recoveries between 86 and 103% indicated that sample matrices had a little effect on the applicability of the suggested approach.

Comparison of the suggested approach with previous methods

The analytical figures of merit (LOD, RSD, and LR) of the introduced approach and other previously published methods[5,7,9,23,24] for determining of the OPPs in different matrices are summarized in Table 4. The presented approach had broader LRs compared to the others. The LODs in the suggested approach were comparable with or less than other approaches. Also, repeatability of introduced approach was acceptable and its RSDs% are better than others. In addition, time of extraction procedure in developed method was less than others. Overall this method is simple, sensitive, efficient, cost benefit, and environmental friendly.

Table 2. The Found concentrations of the selected analytes in the evaluated honey samples. The results are given as mean concentration (ng g^{-1}) ± standard deviation (n=3).

No.	Analyte				
	Parathion–methyl	Triazophos	Parathion	Diazinon	Phoxim
H1	ND [a)]	ND	ND	ND	ND
H2	ND	ND	ND	ND	ND
H3	ND	ND	ND	ND	ND
H4	ND	ND	ND	ND	ND
H5	ND	ND	ND	ND	ND
H6	ND	ND	104 ± 4	ND	ND
H7	ND	ND	ND	ND	ND
H8	ND	ND	ND	ND	ND
H9	ND	ND	ND	ND	ND
H10	ND	ND	ND	ND	ND
H11	ND	ND	ND	ND	ND
H12	ND	ND	ND	ND	ND
H13	ND	ND	ND	ND	ND
H14	ND	ND	ND	73 ± 2	ND
H15	ND	ND	ND	ND	ND
H16	ND	ND	ND	ND	ND
H17	ND	ND	ND	ND	ND
H18	ND	ND	ND	ND	ND
H19	ND	ND	ND	ND	ND
H20	ND	ND	ND	ND	ND
H21	ND	ND	ND	ND	ND

a) Not detected

Table 3. Results of the relative recovery to check the matrix effect for the selected analytes in different honey samples. The analytes' contents were subtracted.

No.	Mean relative recovery ± standard deviation (n=3)				
	Parathion–methyl	Triazophos	Parathion	Diazinon	Phoxim
Samples were spiked with each analyte at a concentration of 30 ng g^{-1}.					
H1	95 ± 2	93 ± 3	93 ± 2	94 ± 1	92 ± 1
H2	89 ± 3	94 ± 1	92 ± 1	92 ± 3	91 ± 2
H3	91 ± 3	94 ± 4	92 ± 4	90 ± 3	91 ± 2
H4	91 ± 4	96 ± 2	89 ± 3	94 ± 1	89 ± 3
H5	92 ± 1	89 ± 4	94 ± 3	89 ± 3	92 ± 1
H6	101 ± 3	96 ± 2	95 ± 1	89 ± 2	86 ± 1
H7	94 ± 3	91 ± 2	91 ± 3	102 ± 4	87 ± 3
H8	95 ± 2	96 ± 3	97 ± 2	94 ± 4	90 ± 2
H9	89 ± 3	100 ± 2	91 ± 3	91 ± 2	90 ± 3
H10	96 ± 2	91 ± 3	93 ± 3	90 ± 3	96 ± 1
H11	93 ± 1	92 ± 3	96 ± 3	102 ± 2	99 ± 3
H12	96 ± 3	102 ± 3	97 ± 3	87 ± 2	94 ± 3
H13	98 ± 2	88 ± 4	93 ± 3	95 ± 3	95 ± 2
H14	102 ± 2	97 ± 2	95 ± 3	93 ± 2	89 ± 1
H15	97 ± 3	93 ± 3	95 ± 3	103 ± 1	89 ± 1
H16	94 ± 2	95 ± 2	93 ± 1	96 ± 3	92 ± 3
H17	90 ± 2	102 ± 1	90 ± 3	94 ± 3	93 ± 2
H18	93 ± 1	96 ± 4	95 ± 2	96 ± 1	95 ± 4
H19	91 ± 3	95 ± 3	89 ± 1	93 ± 4	97 ± 4
H20	94 ± 2	101 ± 3	94 ± 4	96 ± 3	99 ± 3
H21	96 ± 3	94 ± 2	96 ± 4	92 ± 2	97 ± 3
Samples were spiked with each analyte at a concentration of 150 ng g^{-1}.					
H1	93 ± 4	93 ± 3	93 ± 2	93 ± 3	90 ± 1
H2	92 ± 2	92 ± 2	91 ± 2	94 ± 2	89 ± 1
H3	94 ± 3	97 ± 1	95 ± 2	92 ± 3	89 ± 2
H4	89 ± 2	97 ± 3	89 ± 3	92 ± 1	92 ± 4
H5	88 ± 3	91 ± 4	97 ± 2	91 ± 2	89 ± 2
H6	98 ± 2	99 ± 1	98 ± 2	97 ± 3	99 ± 2
H7	97 ± 3	96 ± 4	94 ± 3	103 ± 3	95 ± 3
H8	93 ± 2	94 ± 3	97 ± 3	96 ± 4	92 ± 2
H9	96 ± 3	101 ± 2	93 ± 3	95 ± 2	93 ± 3
H10	98 ± 3	95 ± 3	96 ± 3	94 ± 3	92 ± 2
H11	102 ± 4	94 ± 2	96 ± 1	89 ± 2	89 ± 3
H12	98 ± 4	95 ± 2	91 ± 3	102 ± 3	88 ± 3
H13	96 ± 2	95 ± 3	97 ± 2	96 ± 3	91 ± 4
H14	99 ± 3	101 ± 3	98 ± 3	95 ± 2	90 ± 2
H15	98 ± 3	96 ± 3	95 ± 3	93 ± 2	99 ± 5
H16	102 ± 4	97 ± 2	95 ± 1	89 ± 2	86 ± 1
H17	97 ± 4	93 ± 2	93 ± 3	101 ± 1	87 ± 2
H18	99 ± 3	95 ± 3	98 ± 2	95 ± 2	98 ± 1
H19	95 ± 2	101 ± 2	96 ± 3	91 ± 1	93 ± 2
H20	94 ± 4	93 ± 3	95 ± 3	92 ± 2	95 ± 2
H21	89 ± 1	102 ± 2	94 ± 3	94 ± 3	93 ± 2

Conclusion

A new sample pretreatment method named DSPE–TI–HLLME was introduced for the isolation of some widely used OPPs from honey samples before their determination by HPLC–UV. The obtained outcomes indicated that the introduced approach possess a number of merits including low LODs, an acceptable repeatability, simplicity, and

Table 4. Comparison of the presented method to other methods used for the extraction and determination of the OPPs in food samples.

Method	Sample	LOD [a)] (ng g−1)	LR [b)] (ng g−1)	RSD [c)] (%)	Extraction time (min)	Ref.
UASE–DLLME–SFO–HPLC–UV [d)]	Fruit & Vegetable	1.0–4.0	5.0–800	<9.0	35	5
UA–DLLME–HPLC–UV [e)]	Rice	1.5–3.0	4.0–800	<5.1	~26	7
SFE–SPE–GC–MS [f)]	Wheat & Maize	4.0–53.0	–	<13.0	60	23
QuEChERS–GC–MS [g)]	Peanut oil	0.7–1.6	5.0–200	<8.5	22.5	9
DLLME–SFO–HPLC–UV [h)]	Apple	0.7–2.0	2.0–500	<7.2	25	24
DSPE–TI–HLLME–HPLC–UV [i)]	Honey	0.9–1.7	3.0–5000	<4.0	~20	This method

a) Limit of detection.
b) Linear range.
c) Relative standard deviation.
d) Ultrasonic assisted solvent extraction–dispersive liquid–liquid microextraction–solidification of floating organic drop–high performance liquid chromatography–ultraviolet detection.
e) Ultrasound assisted–dispersive liquid–liquid microextraction–high–performance liquid chromatography–ultraviolet detection.
f) Supercritical fluid extraction–solid phase extraction–gas chromatography–mass spectrometry.
g) Quick easy cheap effective rugged and safe method–gas chromatography–mass spectrometry.
h) Dispersive liquid–liquid microextraction–solidification of floating organic drop–high performance liquid chromatography–ultraviolet detection.
i) Dispersive solid phase extraction–temperature induced–homogenous liquid–liquid microextraction–high performance liquid chromatography–ultraviolet detection.

high EFs and ERs. In addition, it is economical and less hazardous for environment because of low consumption of organic solvents.

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Conflict of interest

The authors declare that they have no conflict of interest.

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