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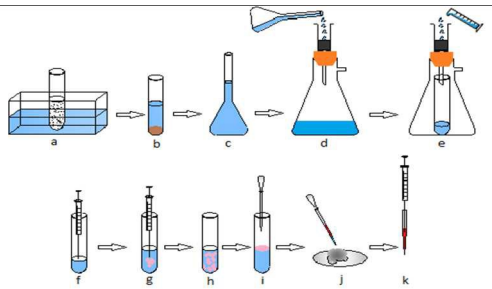


USAL-SPE-LDS-DLLME has been applied for the determination of OPPs in soil samples.

# Ultra-preconcentration and determination of organophosphorus pesticides in soil samples by a combination of ultrasound assisted leaching-solid phase extraction and low-density solvent based dispersive liquid-liquid microextraction

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**Abstract**

An ultra-preconcentration technique composed of ultrasound assisted leaching-solid phase extraction (USAL-SPE) and low-density solvent based dispersive liquid-liquid microextraction (LDS-DLLME) coupled with gas chromatography-mass spectrometry (GC-MS) was developed for preconcentration and determination of organophosphorus pesticides (OPPs) in soil samples. Parameters that affect the efficiency of the procedure were investigated by a fractional factorial design (FFD). Afterwards, variables showing significant effects on the analytical responses were considered using response surface methodology (RSM) based on central composite design (CCD). Under the optimum conditions, the enrichment factors were 7215-9842. The linear range was 0.012-625 ng $g^{-1}$ and limits of detection (LODs) were between 0.002 and 0.125 ng $g^{-1}$. The relative standard deviations (RSDs) were in the range of 5.4-8.3% (n=6). The relative recoveries of OPPs from different soil samples were 84-98%. The proposed methodology constitutes a suitable approach for the analysis of OPPs in complex soil samples requires minimum organic solvents consumption, sample manipulation and increase sample throughput.

## Introduction

Soil is an important component of the ecosystem, and closely related to human survival. As part of the human environment, contaminated soil may cause a serious risk to human health. Organophosphorus pesticides (OPPs) enter the soil ecosystem because of direct spraying on the soil surface during pesticide application in agriculture; the drop from the foliage and stems by the washing of rain and the rotting of plant bodies containing OPPs residues in the soil.[1] These compounds are characterized by their toxicity, relatively high volatility, as well as by their capacity to interfere with cell biochemistry when accumulated in organic tissues in the human body. Pesticides may cause acute anemia, bone structure disorders, teratogenic and embryologic disease, etc.[2,3] So, the analysis of OPPs residue in the soil plays an important role in environmental protection and human health.

Determination of OPPs is usually performed by sample preparation methods coupled with gas chromatography-mass spectrometry (GC-MS),[4-6] gas chromatography-nitrogen phosphorus detector (GC-NPD),[7,8] gas chromatography-flame photometric detector (GC- FPD),[9,10] high performance liquid chromatography-mass spectrometry (HPLC- MS)[11] and high performance liquid chromatography-photodiode array detector (HPLC-DAD).[12,13] Before analysis, due to the complexity of soil sample matrices, their incompatibility with the desired instrumental method and low concentrations of the analytes in soil, a preliminary sample preconcentration and/or separation technique is required. For the determination of OPPs, the separation and preconcentration methods reported in the literature are usually based on supercritical fluid extraction,[2,14] microwave-assisted extraction,[4,15] pressurized liquid extraction,[16,17] ultrasonic extraction[18] and solid phase extraction (SPE),[19] etc. Various configurations of microextraction techniques have frequently been reported as alternatives to the classical approaches in the

literature. Solid phase microextraction[20] and liquid phase microextraction[7,21] are fairly new methods of sample preparation for the preconcentration of OPPs, and have been proved to be simple, inexpensive, fast and virtually solvent-free sample pretreatment techniques with extensive application. Room temperature lixiviation is another method for extracting organic compound from soil samples. It can be assisted by auxiliary energies such as ultrasonic radiation in order to favor the kinetic of the mass-transfer process of the target analytes to the liquid phase. This leads to an increment in the extraction efficiency of the technique in a minimum amount of time. [22,23]

Dispersive liquid–liquid microextraction (DLLME) is an analytical technique recently developed by Rezaee and co-workers.[24] To date, DLLME has been applied for the analysis of various organic and inorganic pollutants in aqueous samples.[25-27] However, there is some inconvenience in retrieving the organic phase. Classical DLLME avoids this problem with the use of extraction organic solvents with densities higher than water, such that, after extraction, the extractant can be sedimentated by centrifugation. To broaden the applicability of DLLME, several recent studies have focused on the use of organic solvents with lower densities than water.[28-32] Very recently, Cabuk and his co-workers proposed a new technique for the collection of an extraction solvent lighter than water after DLLME procedure.[33] After extraction, a disposable glass Pasteur pipette and anhydrous $Na_2SO_4$ (acts as a flow stopper) were used to remove the upper organic extract.

The main disadvantage of the DLLME is that it is not a selective extraction technique and also fails if phases do not separate even after centrifugation (in the case of heavily contaminated extracts). Thus, in order to overcome this problem it is necessary to include a clean-up stage previous to this technique. SPE is widely used as a sample clean-up and preconcentration

technique in sample preparations. Recently, SPE combined with dispersive liquid-liquid microextraction showed a potential ability to isolate the target analytes from the complex samples and reduce the matrix effects.[34,35] This method can provide a very high enrichment factor and high selectivity.[36] The use of SPE after USAL would increase the extraction efficiency of LDS-DLLME technique and extend its applicability to soil samples. To the best of the authors' knowledge, there are no reports about the use of SPE-LDS-DLLME-GC-MS after ultrasound leaching for the analysis of OPPs in soil samples.

In this paper, the USAL-SPE-LDS-DLLME technique is proposed for the extraction and isolation of OPPs from soil, while determination is achieved by GC-MS analysis. To this aim, four OPPs commonly found in environmental samples were selected as target analytes: Diazinon, Chlorpyrifos, Thionazin and *o,o,o*-Triethyl phosphorothioate. The influence of several variables, such as the volume of extraction solvent and disperser solvent, extraction time, salt effect, flow rate of sample solution and volume of sample was studied and optimized with the aid of response surface methodology and experimental design. A fractional factorial design (FFD) was used to screen the significant factors. Then, a central composite design (CCD) was used to conduct a second-order mathematical model relating the enrichment factor with significant independent variables. The optimum conditions were predicted by using the mathematical model and three-dimensional (3D) response surfaces that obtained from it.

## 2. Experimental

### *2.1. Regents*

The standard of OPPs (Diazinon, Chlorpyrifos, Thionazin, *o,o,o*-Triethyl phosphorothioate) were purchased from Sigma- Aldrich (St. Louis, MO, USA). 0.005 g of each analytes (OPPs)

was dissolved in 5.0 mL HPLC grade methanol obtained from Caledon (Ontario, Canada) to prepare a standard solution of 1000 mg $L^{-1}$. All solutions were stored at 4 °C protected from light. 1-dodecan, 1-octanol and toluene as extraction solvents, ethanol, acetone and acetonitrile as disperser solvents were purchased from Merck (Darmstadt, Germany). All other chemicals used were of reagent grade or of the highest purity available. Ultrapure water (18MΩ $cm^{-1}$ resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Plastic and glassware used for the experiments were previously washed with acetone and rinsed carefully with doubly distilled water.

*2.2. Equipment and working conditions*

GC-MS analyses were performed on an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) interfaced to an Agilent 5975C mass selective detector (MSD). The GC–MS system was equipped with a split/splitless injector system and a RXI-5 MS fused silica capillary column with 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness. Helium (purity 99.9999%) was used as the carrier gas at a flow rate of 1 mL $min^{-1}$. The injection port temperature was 260 ºC and used in the splitless mode. The oven temperature program was set up as follows: start at 45 °C, hold for 2 min; increase at 10 °C $min^{-1}$ to 160 °C; increase at 3 °C $min^{-1}$ to 180 °C; and finally increase at 12 °C $min^{-1}$ to 270 °C, hold for 10 min. The ion source, quadrupole, and transfer line temperatures were set to 250 °C, 230 °C, and 280 °C respectively. The MS system was operated in the full-scan mode with a mass range from m/z 45 to 400. The chromatographic peak areas of analytes were identified by comparison with retention time and mass spectra of authentic standards. The analytes were analyzed in selective ion monitoring mode for quantitative determination. The monitored ions of the analytes were selected based on the good

selectivity and high sensitivity, and were set as follows: Diazinon, m/z 179; Chlorpyrifos, m/z 314; Thionazin, m/z 107; *o,o,o*-Triethyl phosphorothioate, m/z 198.

A 40 kHz and 0.138 kW ultrasonic water bath (Tecno-Gaz SpA, Italy) was employed for assisting the ultrasound leaching process. A centrifuge (model Z200A, HERMLE, Germany) was used for centrifuging the mixtures. SPE of OPPs was performed by using 100 mg of $C_{18}$ sorbent with a 3 mL syringe barrel (Waters, Massachusetts, USA).

*2.3. Sampling and sample preparation*

Soil samples were collected from cucumber and cabbage farms (Isfahan, Iran), flat, citrus and greenhouse soils (Rudsar, Iran). The cucumber and cabbage farms were sprayed with the OPPs 5 and 8 days before sampling, respectively. Soil sample used in this study as blank, was collected from an ecological agriculture farm, in which no organophosphorus pesticides had been used during the past three years. The samples were air dried, sieved to size less than 1 mm and kept in airtight amber glass containers at 2 to 4 $^{\circ}$C.

In order to preparation of blank soil samples, 100 g of the ecological agriculture farm soil sample was immersed in 200 mL of methanol, acetone, dichloromethane and n-hexane consecutively and shacked for at least 24 h.[4,37] The treated soil sample was spread out on a tray and air-dried in a fume hood to remove as much solvent as possible. The dried soil sample was stored in an airtight amber glass container at 2 to 4 $^{\circ}$C. The blank soil sample prepared was analyzed before spiking and found no detectable levels of the target compounds.

Fresh spiked soil samples were prepared by weighing two grams of the prepared blank soil sample and spiking with an appropriate amount of OPPs standard solution. The fresh spiked soil

samples were immediately used in USAL-SPE-LDS-DLLME procedure after being air dried waiting for solvent evaporation.

*2.4. The procedure*

A schematic diagram of the extraction procedure is shown in Fig. 1. 2 g fresh spiked soil samples were placed into a 20 mL centrifuge tube, followed by the addition of 2.5 mL of methanol. The mixture was sonicated in a US bath for 2 min. The resulting slurry was centrifuged at 5000 rpm (2906.8 × g rcf) for 5 min and the supernatant liquid was passed through a PTFE syringe filter (13 mm, 0.22 mm) to remove particles.[21] The aliquot of the residual filtrate was placed in a 50 mL cylinder and diluted with double distilled water to 50 mL. Then, the final test portion extract (50 mL) was loaded into a $C_{18}$ SPE sorbent at a flow rate of 15 mL $min^{-1}$ with the aid of a Rotavac vacuum pump (Heidolph, Germany). The $C_{18}$ SPE cartridge was rinsed with 2 mL of double distilled water to remove the matrix interferences. After ventilating of the solid phase, the desired compounds were eluted with 1 mL acetonitrile, which was used as disperser solvent in the subsequent LDS-DLLME procedures. For LDS-DLLME, 10 μL of toluene was added to the SPE acetonitrile extract (1 mL). The resulted mixture was rapidly injected into a 5 mL of double distilled water by using a syringe. A cloudy solution was formed due to the dispersion of the tiny toluene droplets in the aqueous solution. The mixture was centrifuged for 2 min at 5000 rpm (2906.8 × g rcf). By this process, the tiny toluene droplets were floated on the surface of the aqueous solution due to their low density. The organic solvent together with some little aqueous phase was pipetted by using a disposable glass Pasteur pipette. Next, the flow of the aqueous phase was stopped by successive dipping the capillary tip of the pipette into

anhydrous $Na_2SO_4$.[33] The upper organic layer was then removed by using a 10 μL microsyringe and 1 μL of this solution was injected into the GC-MS for analysis.

*Preferred Position for* **Figure 1**

*2.5. Study of experimental variables involved in USAL-SPE-LDS-DLLME*

Different variables can affect the extraction yield in the USAL-SPE-LDS-DLLME procedure and in most case they are correlated. Therefore the optimization through a multivariate approach is recommended. However, some of them might not have a significant effect and can, thus, be obviated. In this respect, a screening step, prior to the optimization step, is helpful in order to assess the significant variables involved in the analytical system under study. In this case, based on the literatures,[34-36] the influence of six variables such as extraction solvent volume, disperser/eluting solvent volume, extraction time (required time for achieving the highest extraction performance), salt effect, flow rate of sample solution through the solid phase and breakthrough volume in SPE, were studied in order to maximize the extraction yield of OPPs in the USAL-SPE-LDS-DLLME procedure. Design generation and statistical analysis were performed using the software package STATGRAPHICS Plus version 5.1 for windows (Rock Vill. MD, USA).

## 3. Results and discussion

In order to obtain the most effective extraction, enrichment factor (EF) was used to evaluate the extraction efficiency under different conditions. The enrichment factor was calculated by using Eq. (1).

$$EF = \frac{C_f}{C_i} \qquad (1)$$

Where EF, $C_f$ and $C_i$ are the enrichment factor, analyte concentration in the final organic phase and initial concentration of analyte, respectively. $C_f$ was calculated by direct injection of OPPs standard solutions in toluene with concentrations in the range of 0.1-1 mg $L^{-1}$.

*3.1. Selection of extraction and disperser/eluting solvent*

Selection of an appropriate extraction and disperser/eluting solvent is of great importance in an USAL-SPE-LDS-DLLME process. The extraction solvent should be selected on the basis of extraction capability of analytes, substantial gas chromatography behavior and low solubility in water. When combining SPE with LDS-DLLME, the eluting solvent of SPE should also play the role of the disperser solvent at the LDS-DLLME stage. The disperser solvent should be soluble in the extraction solvent and miscible in water, thus enabling the formation of fine droplets of extraction solvent in the aqueous phase. Therefore, toluene, 1-octanol and 1-dodecanol as extraction solvent and ethanol, acetone and acetonitrile as disperser/eluting solvent were investigated. In Fig. 2 EF of diazinon extraction by using proposed method is shown for all combinations of disperser/eluting and extraction solvents. This relationship for other OPPs is reported in the supporting information (SI) section (Fig. S1). Regarding the EFs, the combination of toluene as the extraction solvent and acetonitrile as the disperser/eluting solvent is the best and EFs between 7215 and 9842 are attainable.

*Preferred Position for* **Figure 2**

*3.2. Experimental design*

Fractional factorial design (FFD) was employed as a screening design. In this way, the number of experiments was kept low based on the assumption that interaction effects between three or more parameters are small compared to main and two-variable interaction effects. Thus, it is possible to select a fraction of the full fractional design and omit several combinations of parameters from experimental plane.[38, 39] The number of experiments in FFD is given by $2^{k-p}$+C, where k is the number of variables, C number of replicates and p a whole number that indicates how fractionated the experimental design will be. When p is zero, the experimental design is full.[40, 41] The investigated factors and their domains are presented in Table 1. Only two levels were used so that the variables were considered as discrete values and no continuous second-order response model could be estimated. High and low levels of each variable are based on the literatures[34-36] and are donated as -1 and +1.

*Preferred Position for* **Table 1**

The analysis of the results obtained from quarter-fractional factorial design (16 experiments) is visualized using standardized main and two factor interactions effect Pareto charts (P=95%) as are shown in Fig. 3 and SI Fig. S2. As shown, the most important factors affecting the USAL-SPE-LDS-DLLE procedure for OPPs determination are volume of disperser/eluting solvent, volume of extraction solvent and flow rate of sample solution through the solid phase. The other variables (salt effect, extraction time and breakthrough volume in SPE) were not significant factors in the studied range.

*Preferred Position for* **Figure 3**

The central composite face centered design for these three factors was applied in order to optimize the level of effective parameters for improving the efficiency of OPPs extraction by USAL-SPE-LDS-DLLME. The total number of design point needed (N) is determined by the following equation:

$$N = 2^f + 2f + N_0 \quad (2)$$

Where f is the number of variables and $N_0$ is the number of center point.[42] Therefore, totally 20 experiments had to be run for the CCD ($N_0$=6). The low and high levels of these factors were as follow: extraction solvent volume (10-20 μL), dispersive/elution solvent volume (0.5-1.5 mL) and flow rate of sample solution through the solid phase (5-30 mL $min^{-1}$).

Based on the results of the performed experiments the second-order polynomial equation was obtained as shown in Eq. (3).

$$EF = \beta_0 + \beta_1 E + \beta_2 D + \beta_3 F + \beta_{11} E^2 + \beta_{12} ED + \beta_{13} EF + \beta_{22} D^2 + \beta_{23} DF + \beta_{33} F^2 \quad (3)$$

This model consists of three main effects, three two-factor effects and three curvature effects, where the $\beta_0$ is the intercept and the $\beta_1$-$\beta_{33}$ terms represent those parameters of the model which are optimized iteratively to fit, or model the data. The coefficients of determination ($R^2$ and adjusted-$R^2$) were applied to express the quality of fit of the polynomial model equation. $R^2$ is a measure of the amount of variation around the mean explained by the model. The adjusted-$R^2$ is adjusted for the number of terms in the model. It decreases as the number of terms in the model increases, if those additional terms do not add value to the model. The obtained results for these parameters are listed in Table 2.

*Preferred Position for* **Table 2**

In Eq. (3), the positive and the negative coefficients of the main effects show that how the response changes regarding these variables. The absolute value of a coefficient shows the effectiveness of the related effect. For the graphical interpretation of the interactions, the use of three-dimensional (3D) plots of the model is highly recommended.[43-46] Therefore, the results were interpreted based on the 3D graphs obtained from the model. Fig. 4 and Fig. S3 show 3D response surfaces and contour plots of the models. The responses were mapped against two experimental factors while the other factor is held constant at its central level.

*Preferred Position for* **Figure 4**

Fig. 4a and b show that the enrichment factor decreases by increasing the volume of the extraction solvent. This is related to the increase in the volume of floating organic phase. Increasing the floating organic phase volume leads to decrease of concentration of OPPs in the floating phase, therefore, enrichment factor decreases. Fig. 4a and c show that by increasing the disperser/eluting solvent volume in the range of 0.5-1 mL, the efficiency increases. This is because of more efficient elution of the analytes from the SPE cartridge and more properly dispersion of extraction solvent in aqueous solution. But from 1 to 1.5 mL the efficiency decreases. This behavior can be attributed to the increase of OPPs solubility in water. The flow rate of the sample solution through the solid phase is an important factor, because it controls the time of analysis. The flow rate, on the one hand, must be low enough to perform an effective retention of the analytes. On the other hand, it must be high enough not to waste time. As shown in Fig. 4b and c, the maximum enrichment factor was observed in the flow rate of 14-15 mL min$^{-}$

[1]. It was low enough to perform an effective extraction and high enough to shorten the time reasonable.

After the analysis of results, the following conditions were selected as optimal working conditions to evaluate the performance of the extraction procedure for organophosphorus pesticides: 10 μL of toluene as extraction solvent, 1 mL of acetonitrile as dispersive/elution solvent, 1 min as extraction time, 0 w/v% of salt, 15 mL $min^{-1}$ as flow rate of sample solution through the solid phase and 50 mL of sample for SPE.

*3.3. Analytical performance*

The proposed method was evaluated under the optimum condition for the linearity, precision, limits of detection (LODs), limits of quantification (LOQs) and EFs. The results are summarized in Table 3. The linear dynamic ranges (LDRs) were obtained in the range of 0.012-625 ng $g^{-1}$ and the squared regression coefficients ($R^2$) exceeded 0.9931 for all analytes. LODs of the entire method (based on S/N = 3) for the selected analytes ranged from 0.002 to 0.125 ng $g^{-1}$ and LOQs (S/N = 10) from 0.007 to 0.4 ng $g^{-1}$, which seemed quite promising for trace analysis of the analytes in soil sample. The relative standard deviations (RSDs%) for extraction and determination of the analytes were less than 8.3% based on 6 replicates. The enrichment factors were ranged between 7215 and 9842.

*Preferred Position for* **Table 3**

*3.4. Comparison of the proposed method with other methods*

The efficiency of the represented USAL-SPE-LDS-DLLME method was compared with the previously reported methods for determination of OPPs in soil samples. The details of

comparison are summarized in Table 4. In respect to other methods, the proposed method has very low LODs and extremely high EFs. The time required to complete the extraction process, the sample consumption and the linearity obtained by the USAL-SPE-LDS-DLLME are comparable to or better than other reported methods. Also, in our proposed method, low density, low toxicity and more environmental friendly organic solvents were used as extraction solvent. By considering the results, this method proved to be a rapid, sensitive, repeatable and easy to use technique in the determination of OPPs in soil samples.

*Preferred Position for* **Table 4**

*3.5. Soil sample analysis*

The practical applicability of the proposed USAL-SPE-LDS-DLLME method was evaluated under the optimized conditions by extracting the selected organophosphorous pesticides from several soil samples. The concentration of diazinon, chloropyrifos, thionazin and *o,o,o*-Triethyl phosphorothioate in all of the soil samples was detected. To assess matrix effects, the soil samples were spiked with the four OPPs standards at the concentration of 50 ng $g^{-1}$. Three replicate experiments with the whole analysis process were performed and the results are given in Table 5. The relative recovery (RR) was obtained by the following equation:

$$RR = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \qquad (4)$$

Where $C_{found}$, $C_{real}$, and $C_{added}$ are the concentration of analyte after addition of a known amount of standard in the real sample, the concentration of analyte in the real sample and the concentration of known amount of standard which was spiked to the real sample, respectively.

The relative recoveries of the four analytes were satisfactory, in the range between 87.79% and 99.47%. These values of recoveries have confirmed the validity of the proposed method.

*Preferred Position for* **Table 5**

## 4. Conclusion

The proposed analytical methodology based on USAL-SPE-LDS-DLLME technique is an efficient alternative for OPPs determination at trace level in soil samples. The combination of USAL-SPE leaded to an increment of methodology selectivity and sensitivity; and it explains the LDS-DLLME preconcentration capabilities to complex soil samples. Toluene (LDS), which is relatively less toxic in comparison with the widely used chlorinated solvents in DLLME, was successfully used in the present approach. It is important to highlight the experimental design helped to identify and characterize the variables and their interactions that govern the system. This statistical tool also allowed reaching optimum working condition with a minimum number of analytical assays. The main benefits of the proposed methodology for extraction and determination of OPPs were low sample consumption, minimum use of toxic organic solvent, satisfied extraction time, rejection of matrix constituent, simplicity and high enrichment factor. Therefore, the proposed approach can be successfully applied in routine analysis to determine low concentration levels of OPPs in soil samples.

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**Figure Captions**

**Fig. 1.** Schematic diagram of the proposed procedure. (a) Sonication of the mixture of soil sample and methanol (b) Centrifugation of the mixture (c) Filtration and dilution of the aliquot (d) Load of the final test portion extract into a $C_{18}$ SPE cartridge (e) Elution of OPPs with acetonitrile (f) Addition of toluene to the SPE acetonitrile extract (g) Injection of the mixture of toluene (extraction solvent) and acetonitrile (disperser solvent) into a doubled distillated water (h) Cloudy solution (i) Removal of floated extraction solvent after centrifugation by a glass Pasteur pipet (j) Stop the flow of aqueous phase by successive dipping the capillary tip of the pipette into anhydrous $Na_2SO_4$ (j) withdrawal of the organic solvent for the analysis.

**Fig. 2.** Selection of extracting and disperser/eluting solvents in USAL-SPE-LDS-DLLME. USAL conditions: soil sample weight, 2 gr; spiked concentration, 50 ng $g^{-1}$; leaching solvent volume, 2 mL (Methanol); ultrasound radiation time, 2 min; centrifugation time, 5 min; SPE Conditions: water sample volume, 50 mL; flow rate, 15 mL $min^{-1}$; disperser/eluting solvent volume, 1 mL; LDS-DLLME conditions: extraction solvent volume, 10µL; aqueous solution volume, 5 mL; extraction time, 1 min; centrifugation time, 2 min.

**Fig. 3.** Standardized (P = 0.05) Pareto chart, representing the estimated effects of parameters and parameter interactions on enrichment factor.

**Fig. 4.** Estimated response surfaces with related contours for Diazinon by plotting enrichment factor versus a: extraction solvent volume (E, µL) and dispersive solvent volume (D, mL); b: extraction solvent volume (E) and Flow rate of sample solution through solid phase (F, mL $min^{-1}$); c: dispersive solvent volume (D) and Flow rate of sample solution through solid phase (F).

**Fig. 1**

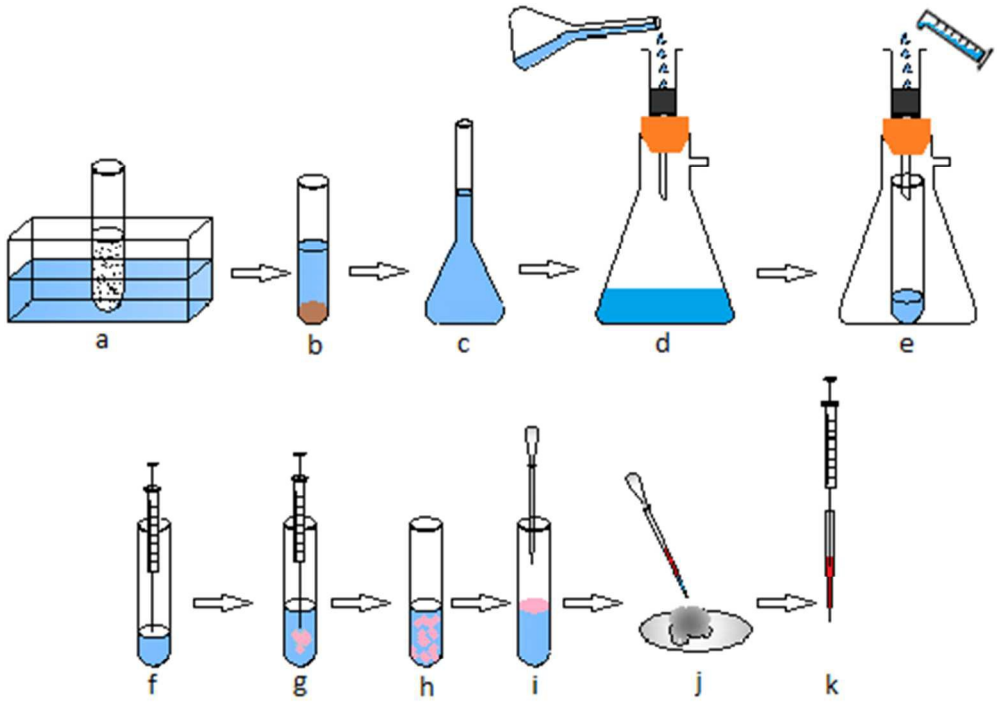

Fig. 2

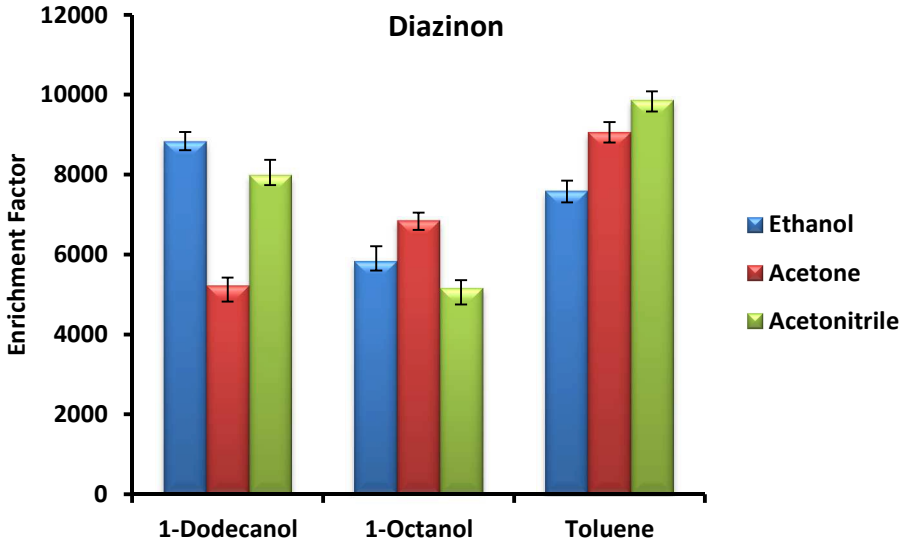

**Fig. 3**

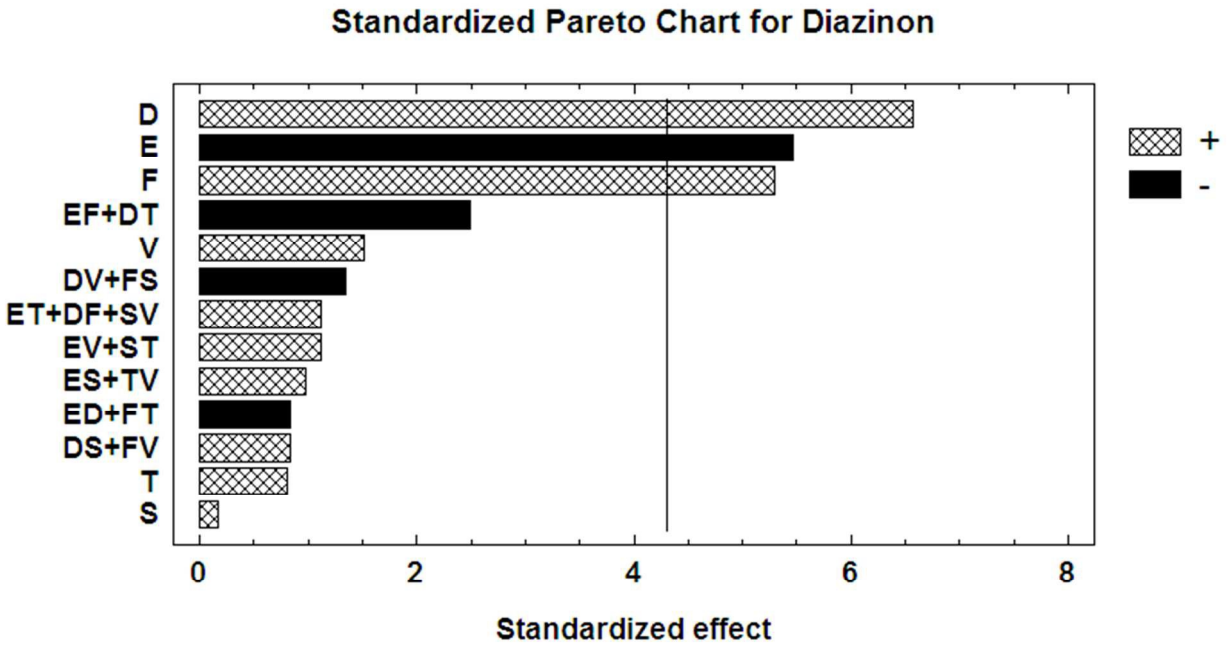

**Fig. 4**

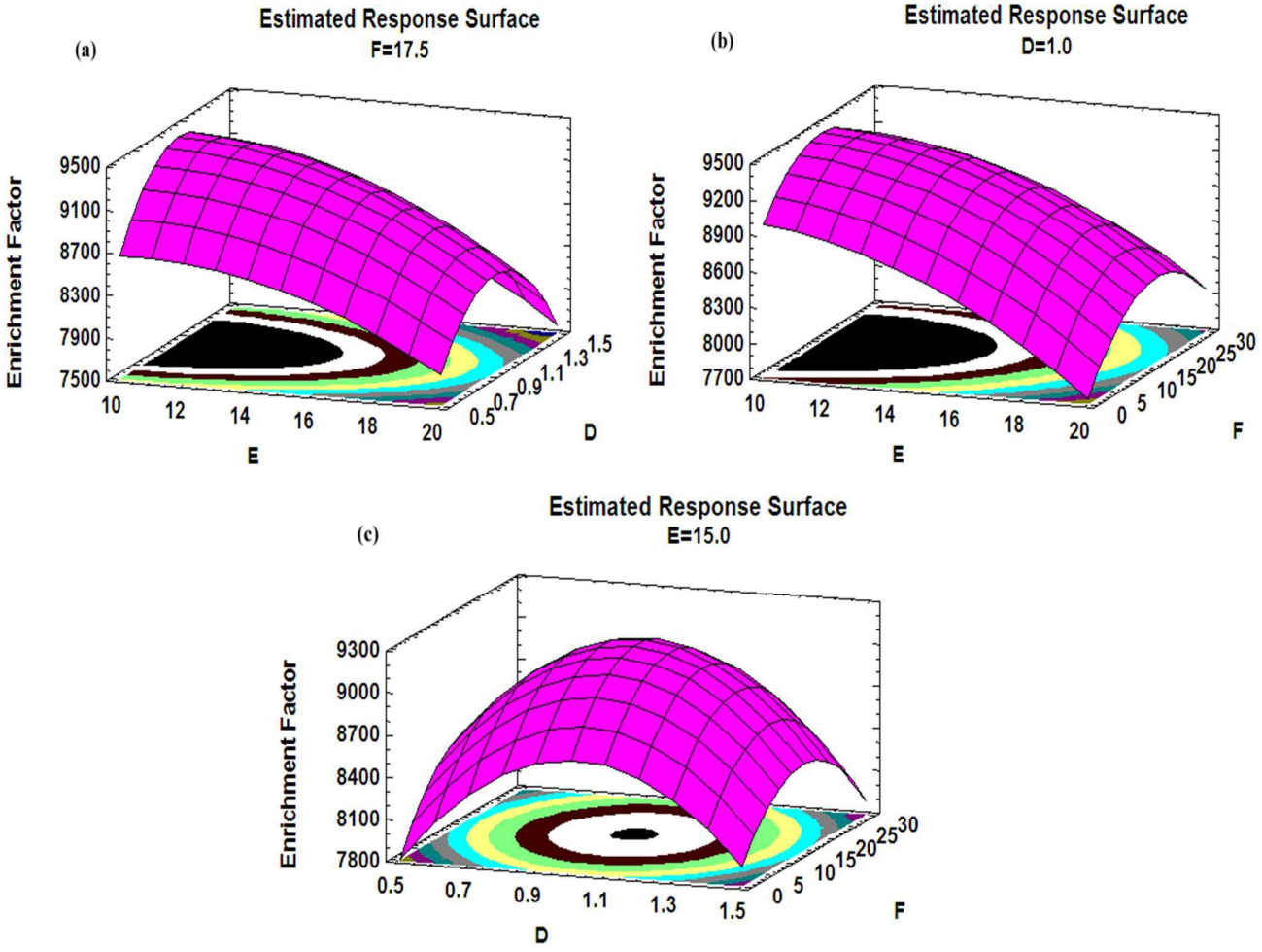

**Table 1**

Investigated variables, their levels and symbols for FFD $2^{6-2}$ design

| Variables | Effect symbol | Variable levels | |
|---|---|---|---|
| | | -1 | +1 |
| Volume of extraction solvent (µL) | E | 10 | 20 |
| Volume of disperser/eluting solvent (mL) | D | 0.5 | 1.5 |
| Extraction time (min) | T | 1 | 30 |
| Salt concentration (w/v %) | S | 0 | 5 |
| flow rate of sample solution through the solid phase (mL $min^{-1}$) | F | 5 | 30 |
| volume of sample solution in SPE (mL) | V | 10 | 200 |

**Table 2**

Coefficient of the regression equation for target analytes

| Coefficients | Diazinon | Chloropyrifos | Thionazin | o,o,o-Triethyl phosphorothioate |
|---|---|---|---|---|
| $\beta_0$ | 4795.34 | 3717.32 | 4550.9 | 5736.92 |
| $\beta_1$ | 193.918 | 246.679 | 60.8162 | 80.6266 |
| $\beta_2$ | 6422.75 | 6370.76 | 4146.62 | 5074.27 |
| $\beta_3$ | 63.0583 | 70.793 | 33.1132 | 44.2482 |
| $\beta_{11}$ | -9.53455 | -11.0516 | -4.5494 | -6.11194 |
| $\beta_{12}$ | -31.26 | -28.7998 | -14.4981 | -6.84763 |
| $\beta_{13}$ | 1.2216 | 1.12546 | 0.558246 | 0.246741 |
| $\beta_{22}$ | -2909.45 | -2907.22 | -1927.34 | -2456.09 |
| $\beta_{23}$ | -11.4 | -10.5028 | -4.9682 | -1.69776 |
| $\beta_{33}$ | -2.18601 | -2.37675 | -1.22509 | -1.60087 |
| $R^2$ | 95.73 | 93.67 | 97.52 | 98.06 |
| Adjusted-$R^2$ | 91.89 | 87.98 | 95.30 | 96.32 |

**Table 3**

Analytical figures of merits of the proposed methodology for OPPs determination in soil samples

| Analyte | Linearity | | LOD | LOQ | Precision | EF[a] |
|---|---|---|---|---|---|---|
| | LDR (ng $g^{-1}$) | $R^2$ | (ng $g^{-1}$) | (ng $g^{-1}$) | (RSD%, n = 6) | |
| Diazinon | 0.012 – 625 | 0.9987 | 0.002 | 0.007 | 7.10 | 9842 |
| Chloropyrifos | 0.025 - 625 | 0.9931 | 0.012 | 0.04 | 6.80 | 8799 |
| Thionazin | 0.25 - 625 | 0.9955 | 0.125 | 0.4 | 8.30 | 7215 |
| *o,o,o*-Triethyl phosphorothioate | 0.025 - 625 | 0.9963 | 0.005 | 0.017 | 5.40 | 9024 |

[a] Extraction factors were calculated based on extraction of 50 ng $g^{-1}$ of each OPPs.

**Table 4**

Characteristic performance data obtained by using USAL-SPE-LDS-DLLME and other techniques in determination of OPPs in soil samples

| Method | LOD[a] (ng $g^{-1}$) | RSD[b] (%) | Enrichmet factor | Time[c] (min) | Sample consumption (g) | LDR (ng $g^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|
| SFE[d]-DLLME-GC-FID | 1-9 | 3.1-7.5 | 97-144 | 40 | 1.2 | 20-8300 | [2] |
| MAE[e]-GC-MS | 0.10-0.12 | 2.5-9.4 | - | 10 | 1 | 1-250 | [4] |
| SDME[f]-GC-NPD | 0.1-2 | 2.1-6.9 | 1.4-12.7 | 11 | 2 | 10-300 | [7] |
| MAE/USAEME[g]-GC-ECD | 0.04-0.13 | 3.1-8.2 | - | 8-9 | 1 | 0.25-10 | [15] |
| MMSPD[h]-GC-NPD | 0.1-0.6 | 1.6-4.5 | - | 60 | 10 | - | [47] |
| USL-SPE-DSLLME[i]-GC-MS | 0.012-0.2 | 4.06-8.9 | 6890-8830 | 15 | 2 | 0.025-625 | [48] |
| USAL-SPE-LDS-DLLME-GC-MS | 0.002-0.125 | 5.4-8.3 | 7215-9842 | 15 | 2 | 0.012-625 | [This work] |

[a] Limit of detection

[b] Relative standard deviation

[c] The required time for completed extraction process

[d] Supercritical fluid extraction

[e] Microwave-assisted extraction

[f] Single drop microextraction

[g] Ultrasound assisted emulsification microextraction

[h] Modified matrix solid-phase dispersion

[i] Ultrasound leaching-solid phase extraction-dispersive-solidification liquid-liquid microextraction

**Table 5**

Determination of OPPs in real and spiked soil samples (n = 3)

| Sample | | 1[a] | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Soil I[b] | Initial concentration[c] | 223.66 | 208.17 | 28.93 | 28.49 |
| | Found[d,e] | 272.87 | 254.98 | 73.91 | 75.58 |
| | Relative recovery (%) | 98.42 | 93.63 | 89.96 | 94.18 |
| | RSD% | 7.9 | 8.2 | 9.7 | 7.6 |
| Soil II | Initial concentration | 175.25 | 172.70 | 14.44 | 7.33 |
| | Found | 219.42 | 219.49 | 64.17 | 53.25 |
| | Relative recovery (%) | 88.34 | 93.58 | 99.47 | 91.85 |
| | RSD% | 6.7 | 5.9 | 6.3 | 5.1 |
| Soil III | Initial concentration | 4.53 | 12.53 | 3.59 | 1.17 |
| | Found | 53.74 | 60.41 | 50.45 | 48.57 |
| | Relative recovery (%) | 98.43 | 95.76 | 93.72 | 94.81 |
| | RSD% | 8.2 | 7.8 | 6.3 | 7.5 |
| Soil IV | Initial concentration | 10.58 | 18.62 | 5.22 | 3.81 |
| | Found | 55.94 | 62.92 | 52.01 | 49.88 |
| | Relative recovery (%) | 90.73 | 88.61 | 93.59 | 92.15 |
| | RSD% | 8.1 | 7.5 | 5.9 | 6.1 |
| Soil V | Initial concentration | 63.67 | 91.01 | 8.72 | 2.49 |
| | Found | 112.47 | 138.80 | 52.61 | 50.02 |
| | Relative recovery (%) | 97.61 | 95.58 | 87.79 | 95.07 |
| | RSD% | 5.8 | 6.3 | 4.2 | 5.7 |

[a] 1: Diazinon; 2: Chloropyrifos; 3: Thionazin; 4: *o,o,o*-Triethyl phosphorothioate.

[b] I: Cucumber farm soil; II: Cabbage farm soil III: Flat soil; IV: Citrus soil; V: Greenhouse soil.

[c] All concentration are in ng $g^{-1}$.

[d] Concentration of OPPs in spiked samples which was found by the proposed method.

[e] 50 ng $g^{-1}$ of each OPPs was spiked in soils.