

# Ultrasound Assisted-Homogeneous Liquid-Liquid Phase Microextraction based on Deep Eutectic Solvents and Ethyl Acetate for Preconcentration of Selected Organochlorine Pesticides in Water Samples

Joseph Matong<sup>1</sup>, Anele Mpupa<sup>1</sup>, Philiswa N. Nomngongo<sup>1\*</sup>

<sup>1</sup> Department of Applied Chemistry, University of Johannesburg, Johannesburg, SOUTH AFRICA

Received 3 March 2018 • Revised 27 June 2018 • Accepted 27 June 2018

## ABSTRACT

A novel and greener methodology for the simultaneous preconcentration of organochlorine pesticides in water samples based on ultrasound assisted-homogeneous liquid-liquid phase microextraction (UA-HLLME) has been developed. Gas chromatography-time of flight mass spectrometry was used for quantification of OCPS in water samples. In this method, choline-chloride-ethylene glycol deep eutectic solvent and ethyl acetate were used as the disperser solvent and extraction solvent, respectively. Univariate and multivariate approaches were used for optimization of the influential parameters that affect the extraction efficiency of the UA-HLLME method. Under the optimum conditions, enrichment factors ranging from 152 to 403 with acceptable recoveries of 85-100% were obtained. The dynamic linear ranges were obtained in the concentration range 0.015–1000  $\mu\text{g L}^{-1}$  with correlation coefficients ranging 0.9952–0.9995. The limits of detection and quantification of the developed UA-HLLME method were in the range 1.9-8.6  $\text{ng L}^{-1}$  and 5.9-26  $\text{ng L}^{-1}$ , respectively. The intra-day and inter-day precision expressed in terms of relative standard deviation (%RSD) ranged from 2.1-4.5% and 3.9-7.3%, respectively. The developed method was successfully applied for the preconcentration and determination of the selected OCPs from 3 different river water samples. The developed procedure displayed simplicity, environmental friendliness, relatively high extraction efficiency, short analysis time and relatively low detection limits.

**Keywords:** organochlorine pesticides, deep eutectic solvents, choline chloride-ethylene glycol mixture, homogeneous liquid-liquid phase microextraction, multivariate approach

## INTRODUCTION

Pesticides have remained in use for several years to control agricultural pests. Hence, their accumulation in the environment is due to their persistence properties [1]. For this reason, high levels of pesticides are still being discovered in numerous environmental media, such as soil and groundwater [2]. Due to unpleasant effects of pesticides on human and environmental health, it is important to identify and quantifying these contaminants in soil and groundwater matrices [1]. The concentrations of these pollutants are usually at trace levels (especially in groundwater) or are trapped within complex matrices (such as soil), which then poses problems of detection and quantification. For this reason, sensitive, reliable and rapid techniques for accurate determination of pesticides in environmental matrices are required.

Chromatographic techniques such as liquid or gas chromatography coupled to different detectors are widely used for the separation and quantification of organochlorine pesticides in different matrices. However, direct analysis of pesticides using these techniques is not suitable. Therefore, sample preparation procedure prior to

chromatographic quantification of pesticides is required. This is done in order to decrease the complexity of the matrix and increase the concentration of pesticides. Conventional sample preparation technique such as liquid-liquid extraction (LLE) and solid phase extraction are the long-standing preconcentration and matrix separation method in analytical chemistry [3]. However, their disadvantages include time-consuming, labor-intensive and need a large amount of toxic organic solvents (especially LLE) [4]. Although SPE uses smaller volumes of potentially toxic solvents as compared to LLE, a significant amount of organic solvents, disposable cartridges, and discs with a special manifold are still required [5]. Recently, liquid/solid phase microextraction techniques which are considered to be relatively green [5] have been developed for extraction of pesticides in different matrices. These include dispersive liquid-liquid microextraction (DLLME) [6,7], single-drop microextraction (SDME) [2] and hollow fiber liquid/solid-phase microextraction (HF-L/SPME) [8,9], among others.

In last decade, a novel sample pretreatment technique known as homogeneous liquid-liquid extraction (HLLE) was developed by Tavakoli et al. [10]. The principle of this technique is similar to other liquid phase based techniques. The HLLE utilizes both the low and high-density solvents. Recently, the same research group has developed miniaturized homogeneous liquid-liquid extraction (MHLLE) and miniaturized counter current liquid-liquid extraction using methanol (co-solvent) and low density solvents (extraction solvent) for determination of pesticides in soil samples [11,12]. These methods combine high throughput analysis, low cost, and environmental sustainability, which is of great importance in analytical chemistry [12]. In MHLLE the choice of suitable disperser or co-solvent is critical. This is to avoid the use of high disperser solvent which may lead to decreased partition coefficients of the analytes between sample and extraction solvent. Therefore, this can be avoided by using environmentally friendly solvents that have similar properties as frequently used solvents such as methanol. Deep eutectic solvents (DESs) have attracted the interest of many researchers as a green alternative solvent type in sample preparation applications. These solvents have attractive properties such as availability of materials at low cost, the ease of synthesis and low toxicity [14-16]. They are obtained by mixing environmentally friendly (or naturally occurring) components that are compatible with each other mostly through hydrogen bonding [14-16].

The aim of this study was to develop and validate an analytical method based on ultrasound assisted-homogeneous liquid-liquid phase microextraction (UA-HLLME) for preconcentration of organochlorine pesticides in river water samples. The applicability of choline chloride-based deep eutectic solvents (DESs) and aprotic solvents was evaluated. The DESs were chosen because of their environmental friendliness. The DES acted only as a dispersion solvent and it assisted the dispersion of extraction solvent (ethyl acetate) within the aqueous solution. It should be noted that after centrifugation step, organic phase (ethyl acetate) was separated while DES remained in the aqueous solution due to its hydrophilic nature. Based on recent literature findings there are only a few publications on using DESs for extraction of organic pollutants [13-15]. Low density solvents were selected because they can be withdrawn directly from the extraction using suitable micro-syringe. The most influential factors affecting the extraction efficiency of analytes were studied using the small central composite design. To the best of our knowledge, there are limited studies or no work at all, on the use of DESs together with ethyl acetate, for the preconcentration of pesticides in water samples.

## EXPERIMENTAL

### Material and Reagents

Pesticide standard mixture EPA 8081 (CRM 46845, 200  $\mu\text{g mL}^{-1}$ ) containing 20 organochlorine pesticides compounds ( $\alpha$ -BHC,  $\delta$ -BHC, lindane,  $\beta$ -BHC,  $\alpha$ -chlordane,  $\gamma$ -chlordane, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin,  $\alpha$ -endosulfan,  $\beta$ -endosulfan, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide and methoxychlor and surrogate compound, decachlorobiphenyl) was supplied by Supelco (Sigma-Aldrich, Bellefonte, PA, USA). Ethyl acetate (HPLC Plus, for HPLC, GC, and residue analysis, 99.9%), oxalic acid (purified grade, 99.999% trace metals basis), choline chloride (>99%), citric acid (ACS reagent,  $\geq 99.5\%$ ), hexane (ACS reagent,  $\geq 99\%$  (GC) and tetrahydrofuran (THF, inhibitor-free, for HPLC,  $\geq 99.9\%$ ) were purchased from Sigma-Aldrich (Bellefonte, PA, USA). A stock solution of OCPs (200  $\mu\text{g L}^{-1}$ ) was prepared in methanol. The working model solutions of lower concentration (50  $\mu\text{g L}^{-1}$ ) were prepared by diluting the stock solution successively with ultra-pure water (Direct-Q® 3UV-R purifier system). The working standard solutions for calibration of GC-MS were prepared by diluting CRM 46845 solutions with hexane.

### Instrumentation

An in-house GC $\times$ GC system consisting of an Agilent 7890 GC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent 7683 auto-sampler and a single-jet liquid nitrogen cryogenic modulator and coupled to a Pegasus 4 dimension time-of-flight mass spectrometer (4D TOF) (LECO Corporation, St. Joseph, MI), operating in the electron ionization (EI) mode. The GC separation was performed using a DB-5 MS [5%-Phenyl]-

methylpolysiloxane] capillary column (30 m × 0.25 mm × 0.25 μm (Agilent Technologies, Wilmington, USA). The injector port temperature was 250°C. The oven temperature was programmed as follows: 90°C (1 min) to 210°C (at 30°C/min) (5 min) to 250°C (at 10°C/min) (4 min) to 300°C (at 30°C/min) (5 min). Splitless injections of 1 μL sample were carried out. Helium was used as carrier gas at 1.0 ml/min. The interface and source temperatures were both set to 290 and 250 °C, respectively, and a solvent delay of 3 min was selected. The GC-TOF-MS, which can acquire up to 500 full-range mass spectra per second, was used with an electron ionization source at 70 eV and detector voltage at -1700 V. The time-of-flight mass spectrometer was operated with 20 spectra/s acquiring the mass range m/z 30–650 and using a multichannel plate voltage of 2700 V. The Chroma TOF 4.32 software was used for data processing and a peak table was obtained with the list of all peaks found. These peaks were matched against NIST library. The minimum similarity match was set at 750. Branson 5800 Ultrasonic Cleaner - 2 1/2 gallon and Eppendorf 5702 Series Centrifuge were used for assisting the extraction procedure and phase separation, respectively.

### Sample Collection and Preparation

River water samples were in Johannesburg South (South Africa) with the geographic coordinates (26°15'58"S 27°51'57"E) respectively. River water was taken from three different streams where there is domestic farming takes place and stored in dark glass containers and taken to the lab for pesticide analysis. It should be noted that the sample analysis was carried out on the same day. Before use, the samples were filtered through 0.45 μm cellulose acetate filters (Millipore HNWP, Bedford, MA, USA) in order to remove suspended particles.

### Synthesis of DES

The synthesis of DES was carried out according to the procedure reported in the literature [16,17]. Choline chloride-ethylene glycol, choline-oxalic acid, and choline-citric acid mixtures at appropriate molar ratios were synthesized and evaluated for extraction of OCPs in water samples. It should be noted that oxalic acid, citric acid, and ethylene glycol were used as a hydrogen bond donors. To describe the procedure briefly, appropriate amounts (in grams) of choline chloride and oxalic acid or citric acid or ethylene glycol were placed in a 50 mL round-bottom flask. The flask was heated on a temperature controlled hot plate stirrer at 80 °C. The mixture of the 2 components was continuously stirred using a magnetic stirrer bar until a homogeneous, colorless liquid formed (about 5-10 min).

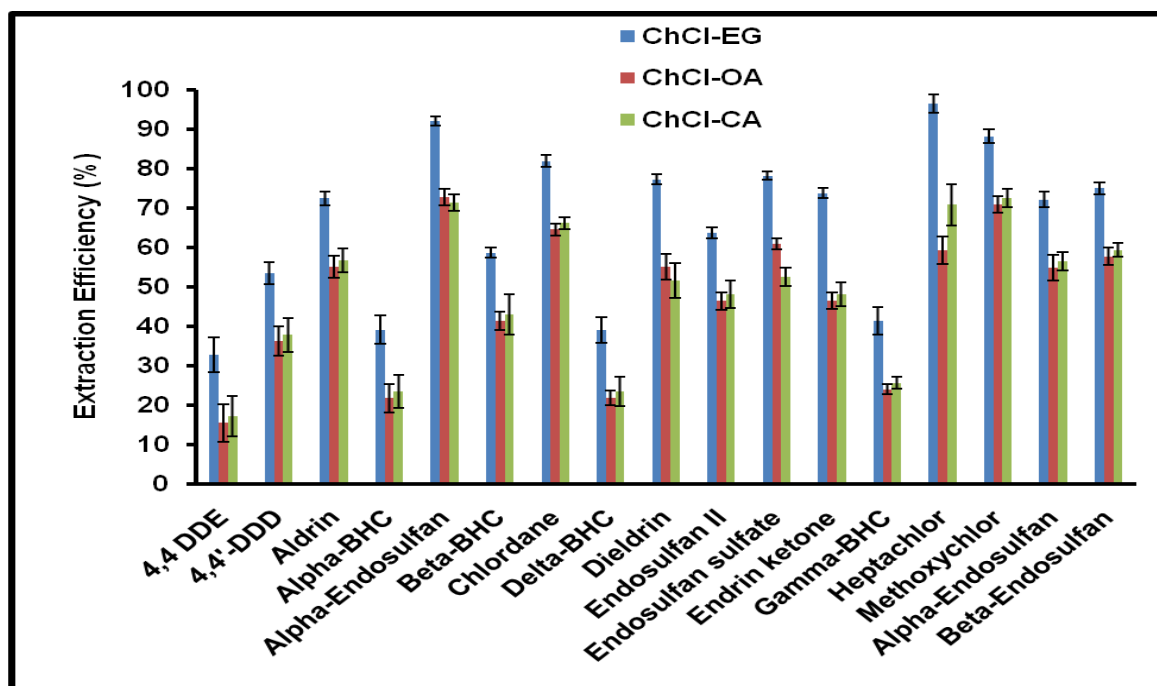
### Ultrasound Assisted-Homogeneous Liquid-Liquid Microextraction Procedure

The UA-HLLME was carried out as follows; 15 mL of an aqueous sample solution containing target analytes (50 μg L<sup>-1</sup>) was placed in a clean dry 25 mL glass sample tube (220 mm (H) × 17 mm (diameter)). An aliquot of 100-500 μL of DES as disperser solvent was added to the aqueous sample solution and a uniform solution was produced. Then, 20-100 μL of a low density solvent was introduced into the uniform solution leading to clustering of DES molecules and as a result, a cloudy solution was produced. So for the solution to entirely disperse the clustered DES droplets into the aqueous phase, the cloudy solution was sonicated for 5-20 min in an ultrasonic bath. At this step, the clustered DES droplets gradually fragmented into small droplets because of the ultrasonic wave involved by short-lived cavitation close to the interface of DES droplets [18]. After centrifugation for 1-10 min at 4400 rpm, two clear phases were observed namely aqueous and organic phases. Then the water immiscible phase was extracted through a micro-syringe and transferred into clean screw-topped centrifuge tube. Furthermore, the acceptor phase (low density organic solvent phase) was diluted to the final volume of 0.2 mL (200 μL) hexane. Then, about 1 μL of the eluent was injected into the GC-TOFMS system for analysis.

## RESULTS AND DISCUSSION

In UA-HLLME, the extraction efficiency is normally influenced by various parameters. These include including the type and the volume of disperser solvent and extraction solvent, extraction time, ionic strength (salt effect), extraction and centrifugation time [19]. The effects of type of extraction solvent and disperser solvent on the extraction of OCPs were optimized using univariate (one-parameter-at-a-time) approach. Other factors were optimized using multivariate approach. The results obtained were expressed in terms of extraction efficiency or extraction recovery which was defined as the percentage of the moles of an analyte ( $n_f$ ) that is transferred into the final organic phase ( $n_i$ ). The percentage extraction efficiency was calculated according to the expression below.

Extraction efficiency (%EE) =  $\frac{C_f \times V_f}{C_i \times V_i} \times 100$ , where  $C_f$  is the amount of the analyte in the acceptor (organic phase), and can be obtained from experimental measurements;  $C_i$  is the initial concentration.



**Figure 1.** Selection of a suitable disperser solvent for extraction OCPs in model aqueous samples solvent. Experimental conditions: aqueous solution volume 15 mL (at  $50 \mu\text{g L}^{-1}$ ); DES volume (250  $\mu\text{L}$ , disperser solvent), ethyl acetate volume (100  $\mu\text{L}$ , extraction), extraction time (20 min), centrifugation time (10 min), ionic strength (3%)

## Effect of Type of Disperser Solvent and Extraction Solvents: Univariate Approach

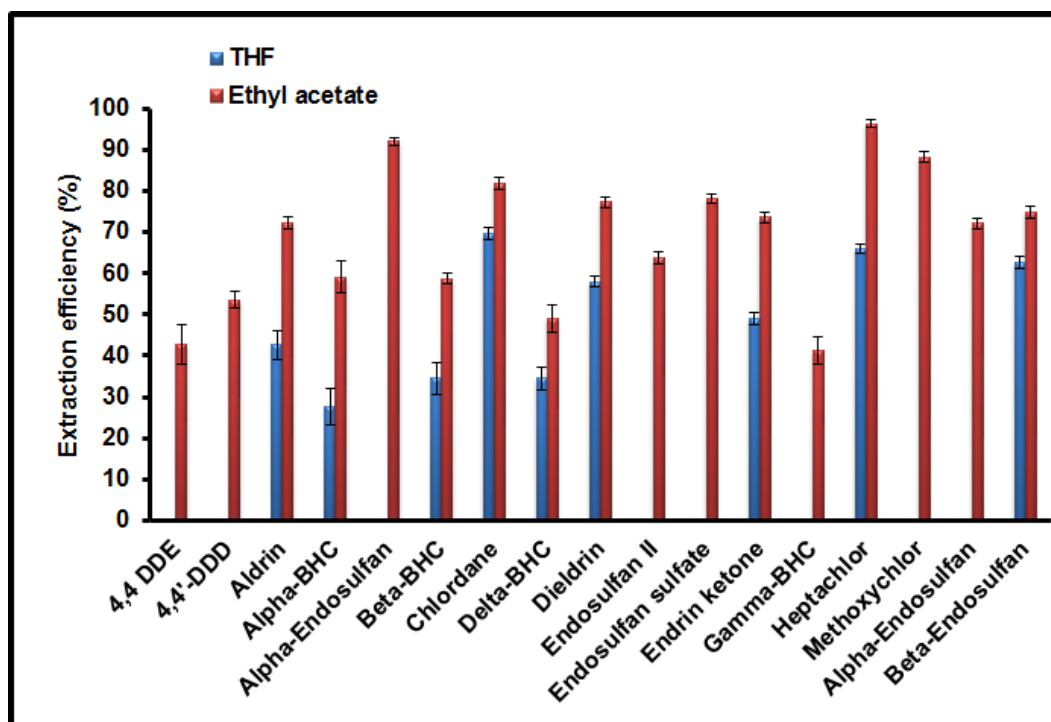
### Selection of disperser solvent

For the UA-HLLME method, the disperser solvent should be soluble with the acceptor phase (organic phase) as well as the donor phase (model and real samples). Therefore, suitability of choline chloride-based DES to be used as disperser solvent was investigated. The DESs investigated include choline chloride-ethylene glycol, choline-oxalic acid, and choline-citric acid. The preconcentration studies were carried out using 15 mL model aqueous samples containing  $50 \mu\text{g L}^{-1}$  OCPs. Ethyl acetate was used as low density solvent. It can be seen from **Figure 1** that higher extraction efficiencies for the studied OCPs were achieved when choline chloride-ethylene glycol was used as the disperser solvent compared to other DESs. This is because clear phase separation was observed when choline chloride-ethylene glycol is used as the disperser solvent. It is worth mentioning that when choline-oxalic acid and choline-citric acid were used, the water immiscibility layer was less distinct compared to choline chloride-ethylene glycol. This might be due to the presence of carboxylic groups which solubilized further the extraction solvent. Therefore, choline chloride-ethylene glycol was chosen a disperser solvent for further experiments.

In view of the results above, the effect of various molar ratios of choline chloride-ethylene glycol (DES1 1:1, DES2 1:2, DES 3 1:3, and DES 1:4) was investigated. The results obtained (**Figure S1**) revealed that quantitative extraction of OCPs was attained when the molar ratio was 1 choline: 1 ethylene glycol. Therefore, the 1 choline chloride: 1 ethylene glycol was selected for further investigations. The 1 choline chloride: 1 ethylene glycol resulted in a free flowing solvent which other were thicker (low viscosity).

### Selection of extraction solvent

The type of extraction solvent is one of the very important factors in obtaining satisfactory extraction efficiency. This is because the physical-chemical properties of the solvents determine its extraction efficiency [20]. In UA-HLLME, the extraction solvent should have a lower density than water, low water solubility and, compatible with analytical detection techniques, environmentally friendly and capable of extracting target analytes [11,19-22]. Therefore, in this study, extraction efficiencies of THF and ethyl acetate for extraction of OCPs in river water were investigated using the UA-HLLME method. The study was carried out using a model sample solution of the analytes at a concentration level of  $50 \mu\text{g L}^{-1}$ . Other parameters were fixed at 250  $\mu\text{L}$ , 100  $\mu\text{L}$ , 20 min, 10 min, 3% for DES volume, extraction solvent volume, extraction time, centrifugation time and ionic strength, respectively. The results obtained are presented in **Figure 2**. It can be seen from these results that relatively higher extraction efficiencies were achieved when using ethyl acetate compared to THF. In addition, only nine pesticides were



**Figure 2.** Selection of a suitable solvent for extraction OCPs in model aqueous samples solvent. Experimental conditions: sample volume 15 mL (at  $50 \mu\text{g L}^{-1}$ ); DES volume (250  $\mu\text{L}$ ), extraction solvent volume (100  $\mu\text{L}$ ), extraction time (20 min), centrifugation time (10 min), ionic strength (3%)

extracted by THF. The findings were attributed to difficulties encountered in order to separate THF from the bulk solution. Ethyl acetate could be separated rapidly and completely and this was in accordance with other studies [11,22]. Therefore, ethyl acetate was selected as an extraction solvent.

### Optimization of the UA-HLLME Operation Parameters: Multivariate Approach

In order to obtain highest extraction efficiency, multivariate optimization of the UA-HLLME method was carried out using small central composite design (SCCD). In the latter, five variables, that is, the volume of extraction and disperser/emulsifier solvents, extraction time, ionic strength and were selected. STATISTICA software was used to estimate the optimum conditions for the performance of the UA-HLLME method. The low and high levels selected for the variables were as follows: DES volume (DESV) (100-500), ethyl acetate volume (EAV) (20-100), ionic strength (IS) (0-10%), extraction time (ET) (5-20 min), and centrifugation time (CT) (1-10 min). Typical design matrix and the response (extraction efficiency, %) are presented in **Table S1**. The analysis of variance (ANOVA) presented in terms of Pareto plot is shown in **Figure S2**. The ANOVA results revealed that all five factors were not significant at 95% confidence level. The response surfaces for factors against the analytical response and quadratic equations were used to estimate the optimum condition. Based on the results obtained, the optimum conditions were; 500  $\mu\text{L}$  of DES as a disperser solvent; 0% (w/v) salt concentration, 100  $\mu\text{L}$  of ethyl acetate as the extraction solvent, 10 minute extraction time and 2 minutes centrifugation time. The performance of the developed method under optimum conditions was evaluated by analyzing triplicates of the model solution. This was done in order to validate the suitability of the estimated optimum conditions. The results obtained ranged from 95-99% and these results demonstrated that the selected conditions were suitable for preconcentration OCPs.

### Analytical Figures of Merit

Under the optimum experimental conditions, the analytical performances of the developed UA-HLLME method preconcentration of OCPs were investigated. The calibration curves were obtained after a set of standard solutions (0 to 1000  $\mu\text{g L}^{-1}$ ) was processed using the UA-HLLME method. The concentrations of the analytes in the eluent solutions (after extraction) were quantified by GC-TOFMS. The limits of detection and quantification were calculated using:  $\text{LOD} = \frac{3 \times Sd}{b}$  and  $\text{LOQ} = \frac{10 \times Sd}{b}$ , respectively, where  $Sd$  is the standard deviation of 12 replicate measurements at lower concentrations of calibration curves and  $b$  is the slope of each calibration curves [15]. Dynamic linear ranges, correlation coefficients, LODs, LOQs and enrichment factors (defined as the ratio between the concentration of the analyte in the final phase ( $C_f$ ) and the initial concentration of the analyte ( $C_i$ ))

**Table 1.** Analytical figures of merit

| Analytes           | Dynamic linear ranges ( $\mu\text{g L}^{-1}$ ) | $R^2$  | LOD ( $\text{ng L}^{-1}$ ) | LOQ ( $\text{ng L}^{-1}$ ) | Precision (%RSD) |           | EF  |
|--------------------|--|--------|----------------------------|----------------------------|------------------|-----------|-----|
|                    |  |        |                            |                            | Intraday         | Inter-day |     |
| 4,4 DDE            | 0.015-850                                      | 0.9991 | 4.8                        | 15                         | 3.8              | 5.2       | 318 |
| 4,4'-DDD           | 0.025-1000                                     | 0.9987 | 7.7                        | 23                         | 2.1              | 4.3       | 322 |
| Aldrin             | 0.020-900                                      | 0.9993 | 5.2                        | 16                         | 4.5              | 5.5       | 260 |
| Alpha-BHC          | 0.020-1000                                     | 0.9978 | 6.7                        | 20                         | 3.9              | 7.0       | 287 |
| Alpha-Endosulfan   | 0.005-950                                      | 0.9953 | 1.9                        | 5.9                        | 4.1              | 6.2       | 253 |
| Beta-BHC           | 0.015-1000                                     | 0.9995 | 3.8                        | 11                         | 3.3              | 5.7       | 165 |
| Chlordane          | 0.020-750                                      | 0.9988 | 6.9                        | 21                         | 4.5              | 5.1       | 152 |
| Delta-BHC          | 0.010-1000                                     | 0.9977 | 3.1                        | 9.4                        | 3.3              | 5.5       | 259 |
| Dieldrin           | 0.015-1000                                     | 0.9985 | 5.4                        | 16                         | 2.2              | 3.9       | 321 |
| Endosulfan II      | 0.025-900                                      | 0.9979 | 8.6                        | 26                         | 3.4              | 4.6       | 351 |
| Endosulfan sulfate | 0.025-950                                      | 0.9992 | 8.6                        | 26                         | 2.1              | 4.9       | 235 |
| Endrin ketone      | 0.020-850                                      | 0.9990 | 6.8                        | 21                         | 2.3              | 5.3       | 403 |
| Gamma-BHC          | 0.015-800                                      | 0.9986 | 6.2                        | 19                         | 3.5              | 6.2       | 285 |
| Heptachlor         | 0.020-1000                                     | 0.9956 | 5.8                        | 18                         | 2.3              | 4.3       | 257 |
| Methoxychlor       | 0.025-1000                                     | 0.9987 | 8.5                        | 26                         | 4.2              | 7.3       | 223 |
| Alpha-Endosulfan   | 0.020-1000                                     | 0.9992 | 7.2                        | 22                         | 3.6              | 6.5       | 247 |
| Beta-Endosulfan    | 0.025-950                                      | 0.9969 | 8.2                        | 25                         | 3.7              | 4.7       | 314 |

**Table 2.** Percentage recoveries of OCPs from spiked double distilled deionized water

| Analytes           | Recovery (%)             |                        |
|--------------------|--------------------------|------------------------|
|                    | 1.0 $\mu\text{g L}^{-1}$ | 5 $\mu\text{g L}^{-1}$ |
| 4,4 DDE            | 97.1 $\pm$ 3.5           | 99.8 $\pm$ 2.6         |
| 4,4'-DDD           | 96.5 $\pm$ 4.8           | 98.9 $\pm$ 1.2         |
| Aldrin             | 96.0 $\pm$ 4.8           | 97.8 $\pm$ 2.0         |
| Alpha-BHC          | 98.5 $\pm$ 4.5           | 99.5 $\pm$ 1.3         |
| Alpha-Endosulfan   | 95.4 $\pm$ 4.1           | 97.6 $\pm$ 2.4         |
| Beta-BHC           | 99.0 $\pm$ 2.9           | 95.9 $\pm$ 3.2         |
| Chlordane          | 98.4 $\pm$ 3.4           | 95.4 $\pm$ 2.6         |
| Delta-BHC          | 98.3 $\pm$ 4.6           | 98.8 $\pm$ 2.9         |
| Dieldrin           | 99.4 $\pm$ 3.7           | 99.4 $\pm$ 4.8         |
| Endosulfan II      | 98.3 $\pm$ 2.3           | 97.7 $\pm$ 3.6         |
| Endosulfan sulfate | 97.7 $\pm$ 2.8           | 99.4 $\pm$ 1.4         |
| Endrin ketone      | 98.9 $\pm$ 2.9           | 98.7 $\pm$ 2.8         |
| Gamma-BHC          | 98.7 $\pm$ 3.3           | 97.7 $\pm$ 3.6         |
| Heptachlor         | 97.5 $\pm$ 3.1           | 99.3 $\pm$ 4.8         |
| Methoxychlor       | 97.8 $\pm$ 2.6           | 99.8 $\pm$ 4.5         |
| Alpha-Endosulfan   | 95.5 $\pm$ 3.8           | 98.3 $\pm$ 5.8         |
| Beta-Endosulfan    | 96.5 $\pm$ 2.1           | 98.6 $\pm$ 4.7         |

within the sample,  $EF = \frac{C_f}{C_i}$  [11] are presented in **Table 1**. The intraday (repeatability, n=15) and interday (reproducibility, n =7) precisions of the developed UA-HLLME method, expressed as the relative standard deviation (%RSD), was determined by carrying out at the 50  $\mu\text{g L}^{-1}$  concentration level and the results are presented in **Table 1**.

The accuracy of the developed method was validated by spiking double distilled deionized water with two concentration levels (1 and 5  $\mu\text{g L}^{-1}$ ). The obtained results of recoveries are given in **Table 2**. According to the analytical results obtained, the percentage recoveries for 1.0  $\mu\text{g L}^{-1}$  ranged from 95.0-99% and for 5.0  $\mu\text{g L}^{-1}$  were between 96% and 99.8%. This demonstrated that developed UA-HLLME method had a potential of extracting and preconcentrating trace OCPs from real samples.

### Comparison of the Proposed Methods with the Other Sample Preparation Techniques

A comparison of the developed method in terms of DLRs, LODs, EF, and RSDs with selected sample preparation reported in the literature for preconcentration of OCPs in different matrices is summarized in **Table 3**. It can be that LODs and DLRs of the UA-HLLME method were better than or comparable with other reported methods except for SPE-DLLME-GC-MS. The EFs were higher than those reported by Refs [12, 23] and comparable those reported by [24]. However, the EFs were lower than those reported by Refs [25-28].

**Table 3.** Comparison of present method with reported liquid-liquid microextraction based methods for the preconcentration of pesticides in different matrices

| Extraction method | Detection system    | DLR ( $\mu\text{g L}^{-1}$ ) | LOD ( $\mu\text{g L}^{-1}$ ) | PF/EF       | RSD (%)  | Ref                 |
|-------------------|---------------------|------------------------------|------------------------------|-------------|----------|---------------------|
| DLLME             | GC-MS               | 0.2–25.0                     | 0.025 -0.88                  | -           | 2-27     | [1]                 |
| PN-SDME           | GC-MS               | 5–500                        | 1.4 -19                      | -           | 4.3-10.2 | [2]                 |
| LDMHLE            | GC/MS               | 0.4–2500                     | 0.13-0.26                    | -           | 4-15     | [11]                |
| MCCLLE            | GC/FID              | 0.4–1000                     | 0.12-0.13                    | 213-221     | 3-13     | [12]                |
| DLLME             | GC-ECD and GC-IT/MS | 0.5-20                       | 0.01–0.2                     | 36-114      | 4-20     | [23]                |
| CCSO-HLLE         | GC-FID              | 1–10000                      | 0.1–5.0                      | 3480–3800   | 2-5      | [24]                |
| SPE-DLLME         | GC-MS               | 0.001–11                     | 0.0005–0.003                 | 3007- 10593 | 3-11     | [25]                |
| MWA-DLLME         | GC-FID              | 3–40000                      | 0.65-1.3                     | 1300-1900   | 4-7      | [26]                |
| DLLME             | GC-NPD              | 0.31-250                     | 0.06-0.17                    | 730-1178    | 3.6-7.4  | [27]                |
| UA-DLLME          | GC-FID              | 1-100                        | 0.09-0.57                    | 315-1153    | <8.0     | [28]                |
| AALLME            | GC-FPD              | 0.5–50                       | 0.02 -0.6                    | -           | 0.4-9.9  | [29]                |
| UA-HLLME          | GC-TOFMS            | 0.015–1000                   | 0.0019-0.0086                | 152- 403    | 3.9-7.3  | <b>Current work</b> |

DLLME = Dispersive liquid-liquid microextraction; PN-SDME = Pneumatic nebulisation single-drop microextraction; MCCLLE = Miniaturized counter current liquid-liquid extraction; CCSO-HLLE = Counter current salting-out homogenous liquid-liquid extraction; LDMHLE= low density miniaturized homogeneous liquid-liquid extraction, SPE-DLLME = Solid phase extraction-dispersive liquid-liquid microextraction method; MWA-DLLME = Microwave-accelerated dispersive liquid-liquid microextraction method; UA-DLLME = Ultrasound assisted dispersive liquid-liquid microextraction; AALLME = Air-assisted liquid-liquid microextraction; UA-HLLME = Ultrasound assisted homogenous liquid-liquid microextraction

**Table 4.** Analytical results from analysis of OCPs in spiked real river samples using UA-HLLME

| Analytes           | RW S1 spiked with $1.0 \mu\text{g L}^{-1}$ |        | RW S2 spiked with $2.0 \mu\text{g L}^{-1}$ |        | RW S2 spiked with $5.0 \mu\text{g L}^{-1}$ |        |
|--------------------|--|--------|--|--------|--|--------|
|                    | Recovery (%)                               | RSD(%) | Recovery (%)                               | RSD(%) | Recovery (%)                               | RSD(%) |
| 4,4 DDE            | 95.8                                       | 3.3    | 90.2                                       | 4.6    | 94.9                                       | 3.8    |
| 4,4'-DDD           | 97.5                                       | 2.5    | 99.1                                       | 3.1    | 96.7                                       | 1.9    |
| Aldrin             | 94.1                                       | 4.7    | 87.2                                       | 2.3    | 96.2                                       | 3.6    |
| Alpha-BHC          | 88.0                                       | 7.2    | 87.9                                       | 4.7    | 96.6                                       | 4.0    |
| Alpha-Endosulfan   | 85.9                                       | 5.6    | 86.9                                       | 5.3    | 96.4                                       | 4.3    |
| Beta-BHC           | 88.0                                       | 3.9    | 97.2                                       | 6.1    | 96.8                                       | 4.0    |
| Chlordane          | 97.9                                       | 4.1    | 97.3                                       | 3.4    | 97.3                                       | 4.7    |
| Delta-BHC          | 87.1                                       | 7.7    | 93.0                                       | 5.0    | 95.5                                       | 2.3    |
| Dieldrin           | 85.7                                       | 6.2    | 96.1                                       | 6.6    | 99.1                                       | 1.9    |
| Endosulfan II      | 88.3                                       | 5.3    | 93.3                                       | 3.1    | 94.4                                       | 4.9    |
| Endosulfan sulfate | 94.4                                       | 3.7    | 91.9                                       | 3.6    | 93.8                                       | 6.0    |
| Endrin ketone      | 95.8                                       | 2.2    | 96.7                                       | 2.3    | 96.5                                       | 5.1    |
| Gamma-BHC          | 98.1                                       | 3.7    | 96.8                                       | 2.6    | 96.7                                       | 4.3    |
| Heptachlor         | 88.7                                       | 6.7    | 92.3                                       | 6.1    | 97.3                                       | 5.8    |
| Methoxychlor       | 87.9                                       | 7.1    | 90.1                                       | 5.6    | 95.1                                       | 3.8    |
| Alpha-Endosulfan   | 85.9                                       | 3.6    | 93.7                                       | 5.2    | 96.0                                       | 2.9    |
| Beta-Endosulfan    | 94.7                                       | 4.5    | 95.3                                       | 2.8    | 99.7                                       | 3.4    |

### Application to Real Water Samples

Real river water samples were analyzed by applying the developed UA-HLLME method. The target analytes were not detected in the river water samples (S1-3). These findings revealed that the OCPs were either not present in river water samples or they were below the detection limits of the developed technique. However, the real samples were then used to investigate the effect of the sample matrix. River water samples S1, S2 and S3 were spiked with the CRM at concentration levels of 1.0, 2.0 and 5  $\mu\text{g L}^{-1}$ , respectively for method validation and the results are summarized in **Table 4** and typical chromatograms are shown in **Figures S4** and **S5**. It can be seen that the recoveries for the all the selected analytes were between 85.7% and 95%. When compared to the results in **Table 4**, these findings suggest that real river water sample matrices have minor to medium interference effects on UA-HLLME. However, the overall results revealed that the developed method is applicable for extraction of trace pesticide residues in water samples. The results obtained in our study, are comparable with other studies reported by [19] in a similar analysis of water for this class of compounds.

### CONCLUSION

A simple, rapid, environmentally friendly, inexpensive and sensitive analytical procedure based on UA-HLLME/GC-MS was developed for the quantification of organochlorine pesticides in river water sample. Factors

affecting the developed method were optimized using univariate and multivariate approaches. The UA-HLLME method displayed relatively wide dynamic linear ranges, low LOD (ng L<sup>-1</sup>) and high preconcentration factors. In addition, UA-HLLME showed relatively good accuracy (in terms of recoveries) and precision (expressed in terms of %RSD). Moreover, developed method was successfully applied for the extraction and preconcentration of the selected OCPs in real water samples.

## ACKNOWLEDGEMENT

The authors wish to thank NRF (grant numbers 98745 & 99270) for financial assistance and Centre for Nanomaterials Science Research for partial funding as well as Applied Chemistry for providing GC-TOFMS facilities.

## REFERENCES

1. Chen B, Wu FQ, Wu WD, Jin BH, Xie LQ, Feng W, Ouyang G. Determination of 27 pesticides in wine by dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. *Microchemical Journal*. 2016;126:415-422. <https://doi.org/10.1016/j.microc.2015.11.003>
2. Wang Y, Wang Z, Zhang H, Shi Y, Ren R, Zhang H, Yu Y. Application of pneumatic nebulization single-drop microextraction for the determination of organophosphorous pesticides by gas chromatography-mass spectrometry. *Journal of separation science*. 2011;34(15):1880-1885. <https://doi.org/10.1002/jssc.201100230>
3. Makahleh A, Yap HF, Saad B. Vortex-assisted liquid-liquid-liquid microextraction (VALLLME) technique: A new microextraction approach for direct liquid chromatography and capillary electrophoresis analysis. *Talanta*. 2015;143:394-401. <https://doi.org/10.1016/j.talanta.2015.05.011>
4. Liang P, Liu G, Wang F, Wang W. Ultrasound-assisted surfactant-enhanced emulsification microextraction with solidification of floating organic droplet followed by high performance liquid chromatography for the determination of strobilurin fungicides in fruit juice samples. *Journal of Chromatography B*. 2013;926:62-67. <https://doi.org/10.1016/j.jchromb.2013.02.011>
5. Lee CH, Shin Y, Nam MW, Jeong KM, Lee J. A new analytical method to determine non-steroidal anti-inflammatory drugs in surface water using in situ derivatization combined with ultrasound-assisted emulsification microextraction followed by gas chromatography-mass spectrometry. *Talanta*. 2014;129:552-559. <https://doi.org/10.1016/j.talanta.2014.06.027>
6. Wu WQ, Chang Q, Wu C, Rao H, Zeng X, Wang C, Wang Z. Ultrasound-assisted surfactant-enhanced emulsification microextraction for the determination of carbamate pesticides in water samples by high performance liquid chromatography. *Journal of Chromatography A*. 2010;1217(11):1773-1778. <https://doi.org/10.1016/j.chroma.2010.01.060>
7. Yan H, Liu B, Du J, Yang G, Row KH. Ultrasound-assisted dispersive liquid-liquid microextraction for the determination of six pyrethroids in river water. *Journal of Chromatography A*. 2010;1217(32):5152-5157. <https://doi.org/10.1016/j.chroma.2010.06.008>
8. Cai CA, Chen G, Qiu J, Jiang R, Zeng F, Zhu F, Ouyang G. Hollow fiber based liquid phase microextraction for the determination of organochlorine pesticides in ecological textiles by gas chromatography-mass spectrometry. *Talanta*. 2016;146:375-380. <https://doi.org/10.1016/j.talanta.2015.08.069>
9. Barahona F, Díaz-Álvarez M, Turiel E, Martín-Esteban A. Molecularly imprinted polymer-coated hollow fiber membrane for the microextraction of triazines directly from environmental waters. *Journal of Chromatography A*. 2016;1442:12-18. <https://doi.org/10.1016/j.chroma.2016.03.004>
10. Tavakoli L, Yamini Y, Ebrahimzadeh H, Shariati S. Homogeneous liquid-liquid extraction for preconcentration of polycyclic aromatic hydrocarbons using a water/methanol/chloroform ternary component system. *Journal of chromatography A*. 2008;1196:133-138. <https://doi.org/10.1016/j.chroma.2008.04.036>
11. Hassan J, Farahani A, Shamsipur M, Damerchili F. Rapid and simple low density miniaturized homogeneous liquid-liquid extraction and gas chromatography/mass spectrometric determination of pesticide residues in sediment. *Journal of hazardous materials*. 2010;184(1-3):869-871. <https://doi.org/10.1016/j.jhazmat.2010.08.008>
12. Hassan J, Sarkouhi M. Miniaturized counter current liquid-liquid extraction for organophosphorus pesticides determination. *Arabian Journal of Chemistry*. 2016;9(1):38-42. <https://doi.org/10.1016/j.arabjc.2013.02.003>
13. Helalat-Nezhad Z, Ghanemi K, Fallah-Mehrjardi M. Dissolution of biological samples in deep eutectic solvents: an approach for extraction of polycyclic aromatic hydrocarbons followed by liquid



- chromatography-fluorescence detection. *Journal of Chromatography A*. 2015;1394:46-53. <https://doi.org/10.1016/j.chroma.2015.03.053>
14. Khezeli T, Daneshfar A, Sahraei R. Emulsification liquid-liquid microextraction based on deep eutectic solvent: an extraction method for the determination of benzene, toluene, ethylbenzene and seven polycyclic aromatic hydrocarbons from water samples. *Journal of Chromatography A*. 2015;1425:25-33. <https://doi.org/10.1016/j.chroma.2015.11.007>
  15. Khezeli T, Daneshfar A, Sahraei R. A green ultrasonic-assisted liquid-liquid microextraction based on deep eutectic solvent for the HPLC-UV determination of ferulic, caffeic and cinnamic acid from olive, almond, sesame and cinnamon oil. *Talanta*. 2016;150:577-585. <https://doi.org/10.1016/j.talanta.2015.12.077>
  16. Khezeli T, Daneshfar A, Sahraei R. A green ultrasonic-assisted liquid-liquid microextraction based on deep eutectic solvent for the HPLC-UV determination of ferulic, caffeic and cinnamic acid from olive, almond, sesame and cinnamon oil. *Talanta*. 2016;150:577-585. <https://doi.org/10.1016/j.talanta.2015.12.077>
  17. Ghanemi K, Navidi MA, Fallah-Mehrdadi M, Dadollahi-Sohrab A. Ultra-fast microwave-assisted digestion in choline chloride-oxalic acid deep eutectic solvent for determining Cu, Fe, Ni and Zn in marine biological samples. *Analytical Methods*. 2014;6(6):1774-1781. <https://doi.org/10.1039/C3AY41843J>
  18. de Castro MD, Priego-Capote F. Lesser known ultrasound-assisted heterogeneous sample-preparation procedures. *TrAC Trends in Analytical Chemistry*. 2007;26(2):154-162. <https://doi.org/10.1016/j.trac.2006.10.008>
  19. Zacharis CK, Tzanavaras PD, Roubos K, Dhima K. Solvent-based de-emulsification dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry for determination of trace organochlorine pesticides in environmental water samples. *Journal of Chromatography A*. 2010;1217(38):5896-5900. <https://doi.org/10.1016/j.chroma.2010.07.065>
  20. Najafi NM, Tavakoli H, Abdollahzadeh Y, Alizadeh R. Comparison of ultrasound-assisted emulsification and dispersive liquid-liquid microextraction methods for the speciation of inorganic selenium in environmental water samples using low density extraction solvents. *Analytica chimica acta*. 2012;714:82-88. <https://doi.org/10.1016/j.aca.2011.11.063>
  21. Shamsipur M, Hassan J. A novel miniaturized homogenous liquid-liquid solvent extraction-high performance liquid chromatographic-fluorescence method for determination of ultra traces of polycyclic aromatic hydrocarbons in sediment samples. *Journal of Chromatography A*. 2010;1217(30):4877-4882. <https://doi.org/10.1016/j.chroma.2010.05.038>
  22. Hassan J. Low density miniaturized homogeneous liquid-liquid extraction: a new high throughput sample preparation technique for the determination of polar pesticides in cow milk. *Journal of analytical chemistry*. 2014;69(9):851-855. <https://doi.org/10.1134/S106193481409007X>
  23. Zacharis CK, Rotsias I, Zachariadis PG, Zotos A. Dispersive liquid-liquid microextraction for the determination of organochlorine pesticides residues in honey by gas chromatography-electron capture and ion trap mass spectrometric detection. *Food chemistry*. 2012;134(3):1665-1672. <https://doi.org/10.1016/j.foodchem.2012.03.073>
  24. Farajzadeh MA, Feriduni B, Mogaddam MR. Development of counter current salting-out homogenous liquid-liquid extraction for isolation and preconcentration of some pesticides from aqueous samples. *Analytica chimica acta*. 2015;885:122-131. <https://doi.org/10.1016/j.aca.2015.05.031>
  25. Shamsipur M, Yazdanfar N, Ghambarian M. Combination of solid-phase extraction with dispersive liquid-liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food chemistry*. 2016;204:289-297. <https://doi.org/10.1016/j.foodchem.2016.02.090>
  26. Farajzadeh MA, Asghari A, Feriduni B. An efficient, rapid and microwave-accelerated dispersive liquid-liquid microextraction method for extraction and pre-concentration of some organophosphorus pesticide residues from aqueous samples. *Journal of Food Composition and Analysis*. 2016;48:73-80. <https://doi.org/10.1016/j.jfca.2016.02.007>
  27. Farajzadeh MA, Mogaddam MR. Acid-base reaction-based dispersive liquid-liquid microextraction method for extraction of three classes of pesticides from fruit juice samples. *Journal of Chromatography A*. 2016;1431:8-16. <https://doi.org/10.1016/j.chroma.2015.12.059>
  28. Cui S, Chen Q, Wang W, Miao J, Wang A, Chen J. Ultra-Preconcentration and Determination of Multiple Pesticide Residues in Water Samples Using Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction and GC-FID. *Chromatographia*. 2013;76(11-12):671-678. <https://doi.org/10.1007/s10337-013-2441-7>
  29. You X, Xing Z, Liu F, Jiang N. Air-assisted liquid-liquid microextraction used for the rapid determination of organophosphorus pesticides in juice samples. *Journal of Chromatography A*. 2013;1311:41-47. <https://doi.org/10.1016/j.chroma.2013.08.080>

Ultrasound assisted-homogeneous liquid-liquid phase microextraction based on deep eutectic solvents and ethyl acetate for preconcentration of selected organochlorine pesticides in water samples

SUPPLEMENTARY DATA

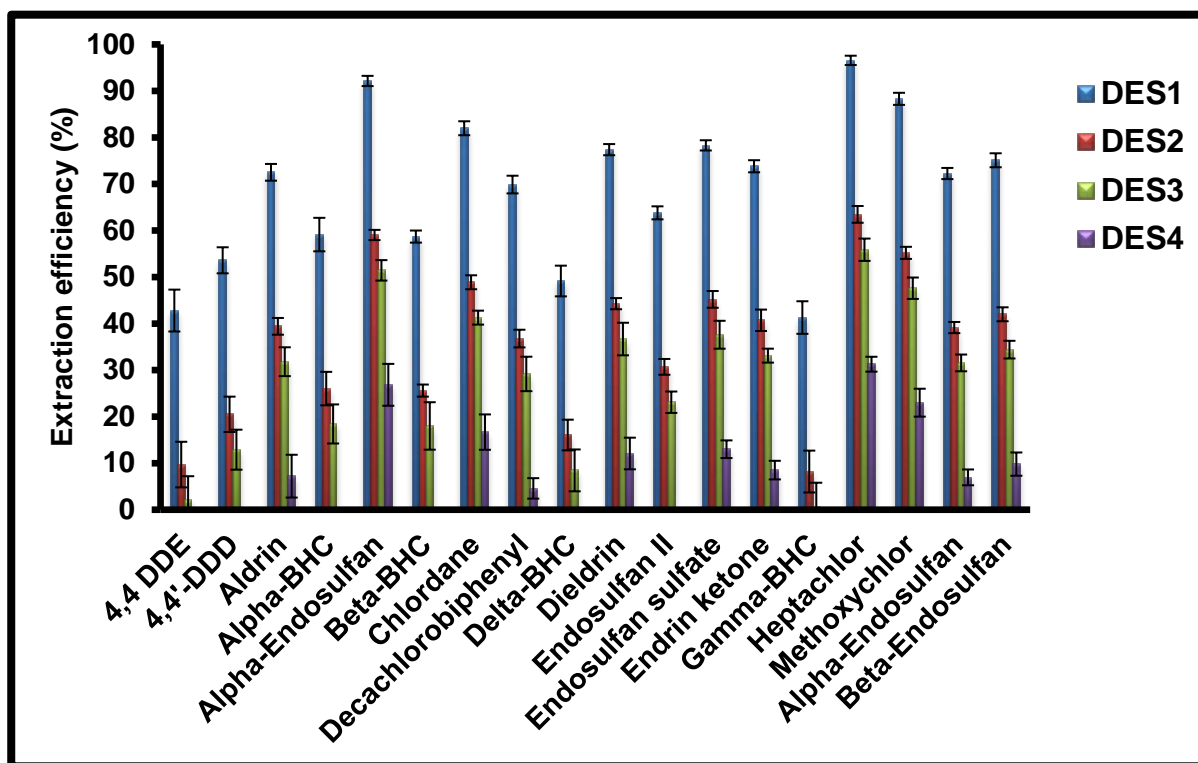
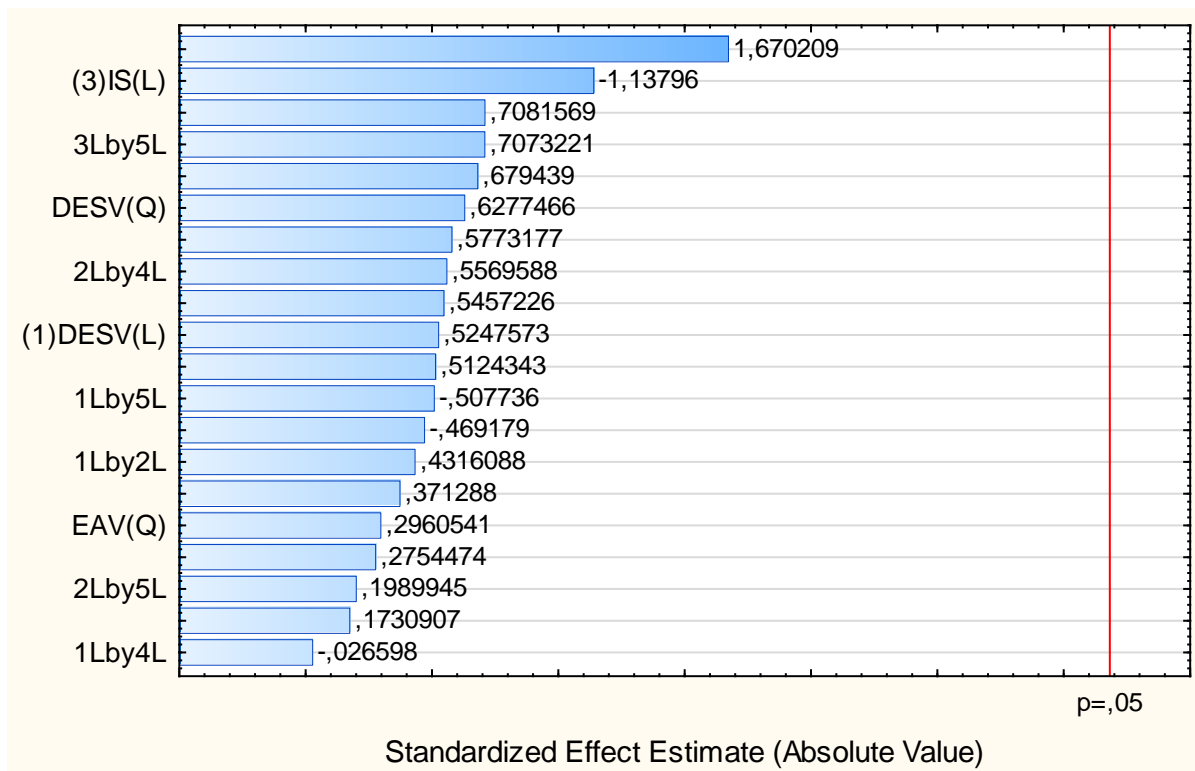


Figure S1. Effect of various molar ratios of choline chloride-ethylene glycol (DES1 1:1, DES2 1:2, DES3 1:3, and DES4 1:4)

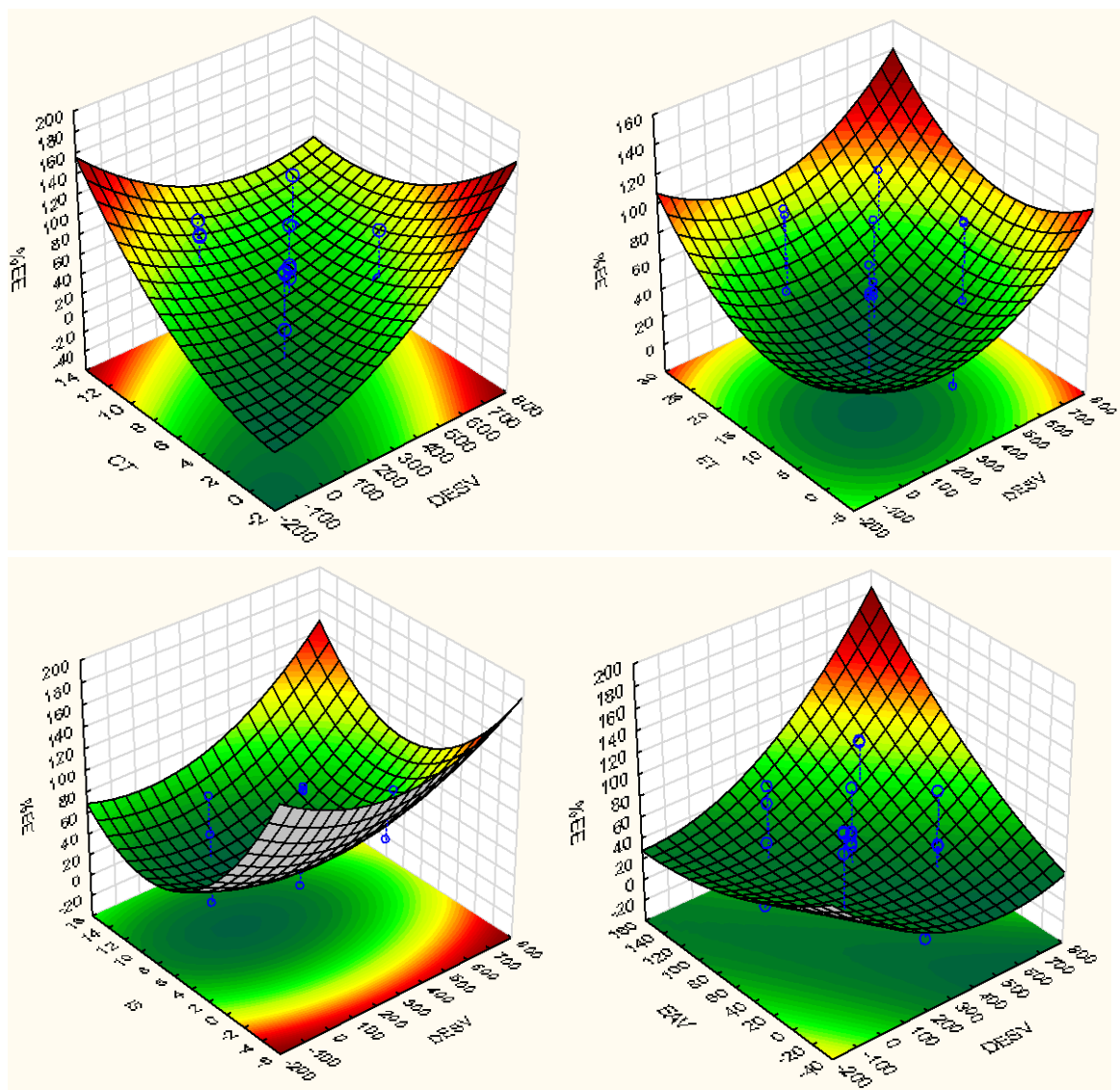
**Table S1.** Design matrix and analytical response

| Runs   | DESV  | EAV   | IS    | ET    | CT     | %EE  |
|--------|-------|-------|-------|-------|--------|------|
| 1      | 500   | 100   | 0     | 20    | 10     | 98.3 |
| 2      | 500   | 20    | 10    | 20    | 10     | 45.3 |
| 3      | 100   | 100   | 10    | 20    | 3      | 41.3 |
| 4      | 500   | 100   | 10    | 5     | 3      | 43.9 |
| 5      | 500   | 100   | 0     | 5     | 3      | 97.7 |
| 6      | 500   | 20    | 0     | 5     | 10     | 98.3 |
| 7      | 100   | 20    | 0     | 20    | 3      | 99.1 |
| 8      | 100   | 20    | 10    | 5     | 10     | 78.9 |
| 9      | 100   | 100   | 0     | 20    | 10     | 94.1 |
| 10     | 500   | 20    | 10    | 20    | 3      | 47.8 |
| 11     | 100   | 100   | 10    | 5     | 10     | 77.6 |
| 12     | 100   | 20    | 0     | 5     | 3      | 97.9 |
| 13     | -72.2 | 60    | 5     | 12.5  | 6.5    | 23.3 |
| 14     | 672   | 60    | 5     | 12.5  | 6.5    | 42.1 |
| 15     | 300   | -14.4 | 5     | 12.5  | 6.5    | 0.00 |
| 16     | 300   | 134   | 5     | 12.5  | 6.5    | 42.3 |
| 17     | 300   | 60    | -4.31 | 12.5  | 6.5    | 95.9 |
| 18     | 300   | 60    | 14.3  | 12.5  | 6.5    | 42.1 |
| 19     | 300   | 60    | 5     | -1.46 | 6.5    | 15.3 |
| 20     | 300   | 60    | 5     | 26.5  | 6.5    | 55.7 |
| 21     | 300   | 60    | 5     | 12.5  | -0.014 | 23.6 |
| 22     | 300   | 60    | 5     | 12.5  | 13.0   | 45.4 |
| 23 (C) | 300   | 60    | 5     | 12.5  | 6.5    | 52.3 |
| 24 (C) | 300   | 60    | 5     | 12.5  | 6.5    | 52.4 |

DESV volume (DESV), ethyl acetate volume (EAV), ionic strength (IS), extraction time (ET) and centrifugation time (CT)



**Figure S2.** Typical Pareto charts for optimization of ultrasound assisted-homogeneous liquid-liquid phase microextraction of pesticides



**Figure S3.** Typical response surface curves for optimization of ultrasound assisted-homogeneous liquid-liquid phase microextraction of pesticides

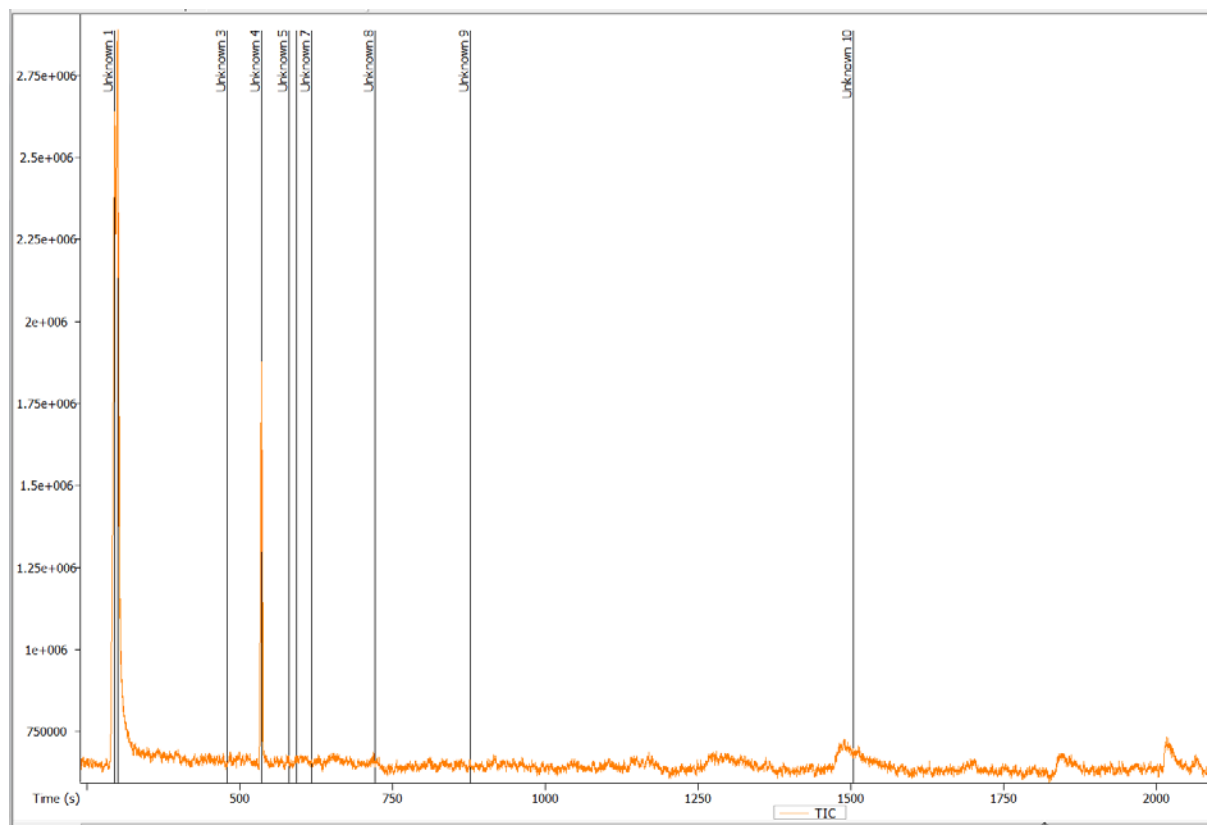


Figure S4. Typical gas chromatogram: Unspiked sample

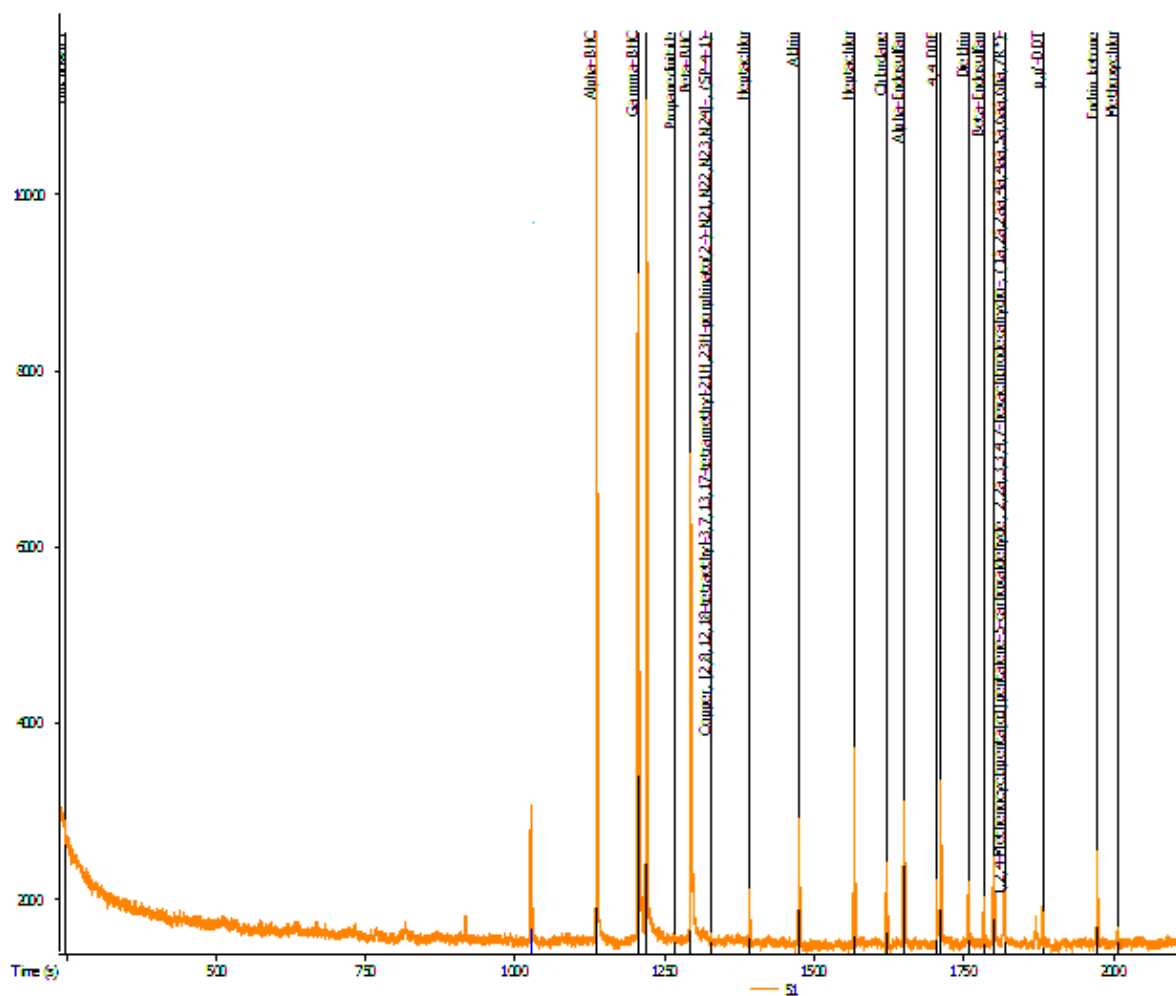


Figure S5. Typical gas chromatogram: spiked sample

<http://www.eurasianjournals.com>