

Determination of organochlorine pesticides in river water samples by liquid-liquid-liquid extraction combined with dispersive liquid-liquid microextraction

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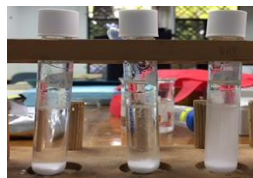
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Article history :

Received 15 November 2018

Accepted 9 December 2018

GRAPHICAL ABSTRACT



Formation of three phases system in LLE: aqueous phase (bottom), acetonitrile phase (middle) and n-hexane (top)

ABSTRACT

Aldrin and dieldrin are insecticides of organochlorine pesticides (OCPs), widely used to kill pest and known to be highly persistent and exhibit slow degradation. Leaching from agricultural runoff causes them to enter nearby water bodies, which can pose a threat to humans and aquatic organism. Determination of OCPs in Sungai Skudai is crucial because the river serves as the raw water source for part of Johor drinking water. A new extraction technique termed liquid-liquid-liquid extraction (LLE) combined with dispersive liquid-liquid microextraction (DLLME) followed by gas chromatograph-microelectron capture detector (GC- μ ECD) was applied to determine aldrin and dieldrin in river water samples of Sungai Skudai. LLE-DLLME involves three phases which are water sample, acetonitrile (ACN) and n-hexane (clean-up solvent). Targeted pesticides with medium polarity were extracted into the ACN phase, non-polar interference into n-hexane, and polar compound remained in the sample. The ACN phase is separated from aqueous phase using sodium sulfate as a phase separating agent. Calibration curves for aldrin and dieldrin were linear in the range of 2-20 μ g/L with a good coefficient of determination, r^2 of 0.9995 and 0.9985, respectively. The percentage recovery obtained for aldrin and dieldrin were 39% and 76% with relative standard deviation (RSD) of 15.97 and 9.81%, respectively. Analysis on Sungai Skudai river water samples revealed that aldrin was present at concentration of 0.27 μ g/L while dieldrin at 0.089 μ g/L. Both pesticide concentrations were above the standard set for raw water quality by Ministry of Health Malaysia and World Health Organization (WHO) for drinking water purpose which is 0.03 μ g/L for combined aldrin and dieldrin. In summary, the LLE-DLLME method is simple, inexpensive and allows extraction of samples without the need of filtration to remove suspended solids.

Keywords: Aldrin, dieldrin, LLE-DLLME, GC- μ ECD, river water analysis

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1. INTRODUCTION

Organochlorine pesticides (OCPs) are man-made chemicals that are widely used in the agriculture industry to kill insects. OCPs are known as highly toxic, persistent and have a tendency to bio-accumulate. Many countries in the world had banned the use of OCPs due to their negative effects but their presence can still be detected in environment such as surface water and ground water [1]. The sources of OCPs in the aquatic ecosystem are from domestic sewage, industrial wastewater discharges and agricultural runoff [2]. Determination of OCPs in surface water that are destined for human consumption is important as its presence is detrimental to human health even at a very low concentration. Different legislations have established guidelines for the maximum allowable concentration of OCPs in water for human consumption. The recommended raw water quality set by the Ministry of Health Malaysia and (World Health Organization (WHO) on drinking water quality guideline for aldrin and dieldrin are 0.03 μ g/L [3, 4].

The presences of these contaminants in environmental samples are often in very low concentrations therefore their detection can be very difficult [5]. Efficient and selective techniques are therefore required to detect them in water. There are several techniques employed in extraction of these contaminants from environmental samples. The most common techniques are liquid-liquid extraction (LLE) and solid phase extraction (SPE). However, both of these techniques have several drawbacks for example in LLE, it requires large amount of organic solvents which does not meet the current demands of sample preparation that is moving towards greener chemistry [6].

A new method was developed by Farajzadeh and coworkers on extraction of pesticides in samples that contain high content of solid without filtration [7]. The developed method is the combination of liquid-liquid-liquid extraction (LLE) and dispersive liquid-liquid microextraction (DLLME.) LLE is an extraction involving three phases system consisting of polar aqueous phase for water sample, non-polar hexane for clean-up purpose and

moderately polar acetonitrile which is for the target pesticides. Separation occurs with polar and ionic compound remain in the aqueous phase, non-polar interference goes into hexane and semi-polar compound extracted into the semi-polar solvents. The main advantage of this method is that the samples do not need to be filtered before the analysis, therefore for river samples that contain high TSS, the analyte of interest that might be adsorbed onto the suspended solid can be extracted as well. This study utilized the new techniques based on LLE with DLLME on extraction of OCPs named aldrin and dieldrin in water samples from Sungai Skudai without filtering the samples. Several parameters affecting extraction efficiencies were investigated and optimized. A quantitative analysis of pesticides was carried out using gas chromatograph with microelectron capture detector (GC- μ ECD).

2. EXPERIMENTAL

Peak identification for pesticides studied was carried out by injecting stock solution of aldrin and mixtures standards solution containing aldrin and dieldrin at 20 μ g/L. Then, the analytical method was first validated by establishing calibration curves of mixtures standards in the range of concentration 2-20 μ g/L. The mixture standard was injected into GC three times in a day to evaluate the performance of the detector. Then, optimization of LLE-DLLME was carried out on ultrapure water spiked with mixtures standards containing the analyte.

First, 5 mL of ultrapure water containing the analytes was placed in 10 mL screw cap glass test tube. 1.4 mL acetonitrile (ACN) was added and vortexed for 30 seconds. Then, 1 mL of n-hexane was added and vortexed for another 30 seconds. n-hexane phase was formed on the surface of the water due to its low density. Then, 2.5 g of anhydrous sodium sulfate, Na_2SO_4 was added to separate the ACN phase from aqueous phase. The mixture was vortexed for 2 minutes to allow dissolution of the salt. Then, it was centrifuged at 4000 rpm for 5 minutes and three phases was achieved; aqueous, acetonitrile and hexane phases. Separated ACN phase was collected with 1 mL syringe, mixed with 40 μ L carbon tetrachloride (CCl_4) and rapidly injected into 5 mL ultrapure water where cloudy solution was formed from the dispersion of CCl_4 in the water. The mixture was then centrifuged at 2500 rpm for 5 minutes which resulted in sedimentation of the dispersed droplets. The dispersed droplet formed on the bottom of the tube was collected with 1 μ L syringe and subjected to solvent evaporation for one day. The extractant was reconstituted with n-hexane and injected into GC- μ ECD for quantitative analysis. The optimized conditions for LLE-DLLME was then applied to determine aldrin and dieldrin in river water samples. Recovery study was performed by spiking the sample with mixture standards.

Quantitative analysis of aldrin and dieldrin was performed using Agilent 7890B gas chromatograph (Agilent, USA), equipped with a μ - ^{63}Ni electron capture detector (GC- μ ECD) and an HP-5MS fused silica capillary column (30 m \times 0.32 mm \times 0.25 μ m (J&W Scientific, Folsom, USA). Helium was used as carrier gas with a flow rate of 1.5 mL /min. Nitrogen was used as make-up gas at a rate of 60 mL/min. The temperature of injector and detector were kept at 250°C and 320°C, respectively. The oven temperature was programmed as follow: 60 to 170°C at a rate of 10°C/min, to 280°C (3 min hold) at a rate of 5°C/min, and finally to 300°C at the rate of 15°C/min. The injection volume was 1 μ L. All injections were made in splitless mode. Temperature of detector: 320°C.

3. RESULTS AND DISCUSSION

3.1 Peak identification of aldrin and dieldrin

Direct injection of standard solution of aldrin showed its retention time of 16.63 min. Injection of mixtures standards containing aldrin and dieldrin shows retention time of 20.21 min for dieldrin. Figure 1 shows chromatogram obtained with mixtures standards at 20 μ g/L. The chromatogram shows both pesticides have good resolution and sharp peak shapes.

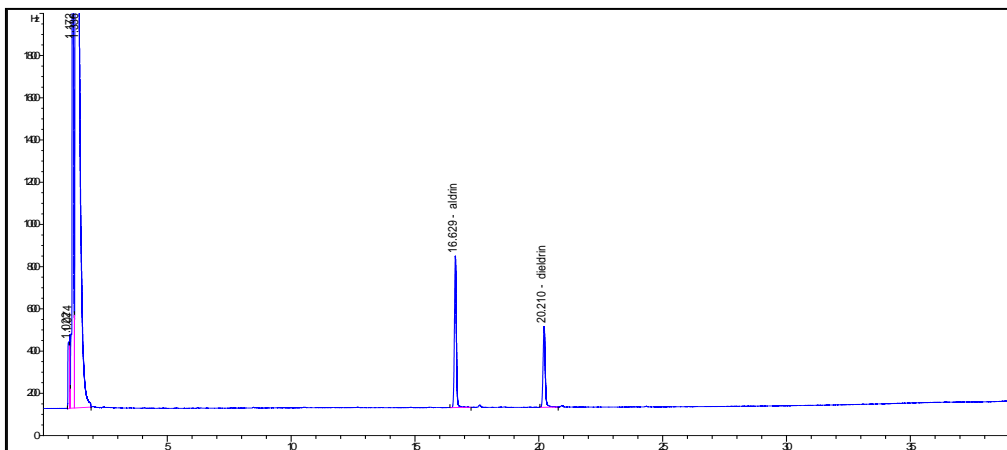


Figure 1 Chromatogram of mixture standards containing aldrin and dieldrin at 20 µg/L.

3.2 Method validation

Figure 2 shows calibration curves for studied OCPs. Plots of peak area against concentration of standards yield a linear graph. Calculated calibration curves gave good linearity for aldrin and dieldrin in the low concentration range of 2-20 µg/L with coefficient of determination, R^2 of 0.9995 and 0.9985, respectively.

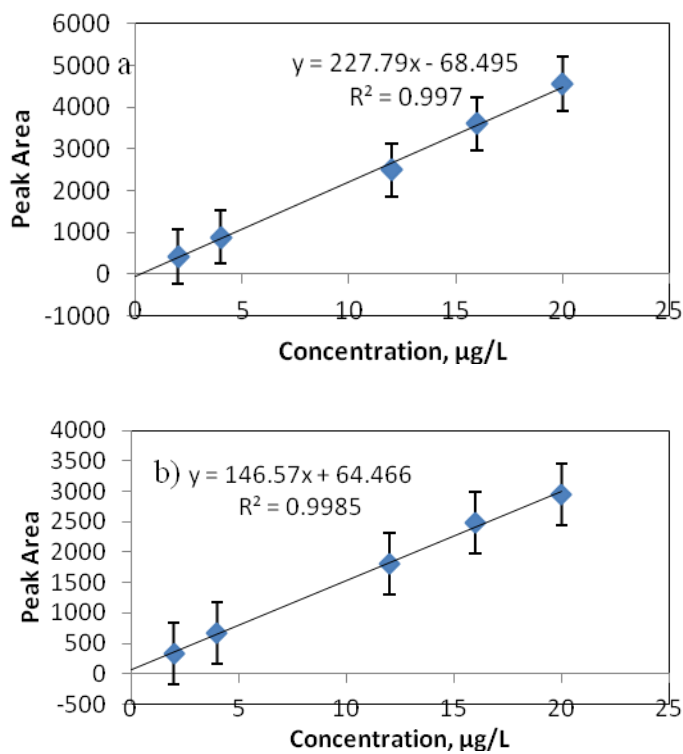


Figure 2 Calibration curves for standard solutions of aldrin (top) and dieldrin (bottom).

LOD and LOQ were calculated based on calibration curves obtained. LOD obtained for aldrin and dieldrin were 1.45 $\mu\text{g/L}$ and 1.02 $\mu\text{g/L}$, respectively. Better LOD are obtained since GC- μECD is a sensitive detector for halogenated pesticides. LOQ for aldrin and dieldrin are 4.83 $\mu\text{g/L}$ and 3.39 $\mu\text{g/L}$, respectively.

3.3 Optimization of LLE-DLLME

In LLE, several factors need to be taken into consideration in order to achieve the three phases system. After the three phases system was achieved, some parameters were then being optimized such as volume of extraction solvent, volume of clean-up solvent, type and volume of pre-concentration solvent. For the purpose of this research, each parameter was tested and optimized one at a time.

Volume of extraction solvent. In LLE, different volume of acetonitrile was examined (1.3, 1.4, 1.5 and 1.6 mL). It was observed that when volume of acetonitrile is increased, the volume of sedimented phase collected in DLLME procedure decreased. Additionally, less volume of acetonitrile used resulted in higher signal of analyte. Finally, 1.4 mL of ACN was selected as the most optimum volume.

Volume of n-hexane. n-hexane act as clean-up solvent to remove non-polar interference in samples. Volume of n-hexane was optimized to see its effect on analyte signal. Significant volume of n-hexane might cause the targeted pesticides to be extracted into n-hexane since dieldrin has medium-low polarity. From 1-2 mL volume of n-hexane studied, 1 mL gave higher signal of analyte. Therefore, 1 mL was used.

Type of pre-concentration solvent. Pre-concentration solvent used in DLLME need to meet important criteria such as lower density than water, formed cloudy solution upon dispersion into aqueous phase, insoluble in water and able to pre-concentrate the targeted pesticides. Three solvents were tested named chloroform, dichloromethane and carbon tetrachloride. Among these solvents, only CCl_4 form cloudy solution when dispersed in aqueous phase and form sedimented phase on the bottom of the test tube. Therefore, it was chosen as pre-concentration solvent in DLLME.

Volume of pre-concentration solvent. Different volumes of CCl_4 were studied in order to observe its effect on the extraction efficiencies (20, 30, 35, 40 and 45 μL). Results show that higher signal of analyte was obtained with 40 μL of CCl_4 . No sedimented phase was obtained when low volume of CCl_4 was used. 40 μL was finally selected.

3.4 Analysis of river water samples.

The optimized LLE-DLLME was applied on river water samples of Sungai Skudai to determine the presence and concentration of aldrin and dieldrin. The river water samples taken were not filtered before analysis to remove the suspended solid in the samples because there is a possibility that the targeted pesticides are adsorbed onto the suspended solid. The unfiltered samples were analyzed directly following the procedure described in Section 2. Quantitative analysis was carried out with GC- μECD . The samples were analyzed in triplicate measurements.

The calculated concentrations were 25.58 $\mu\text{g/L}$ for aldrin and 8.44 $\mu\text{g/L}$ for dieldrin. After correcting with pre-concentration factor, the actual concentration of aldrin and dieldrin were 0.27 $\mu\text{g/L}$ and 0.089 $\mu\text{g/L}$, respectively. The %RSD obtained for aldrin was 15.97% and 9.81% for dieldrin. Figure 3 (A) shows the chromatogram obtained for the water samples. The presence of other peaks in the chromatogram showed that the method is able to extract other contaminants in the water samples. However, only the targeted pesticides were quantitated.

Recovery study was performed by spiking the water samples with 0.6 $\mu\text{g/L}$ mixture of aldrin and dieldrin to evaluate the efficiency of LLE-DLLME and possible matrix effect. Figure 3 (B) shows chromatogram obtained with spiked water samples. Percentage recoveries obtained for aldrin and dieldrin were 39 and 76%, respectively. Recovery for aldrin obtained was rather low while satisfactory recovery was obtained with dieldrin. Low recovery of aldrin was possibly because some steps involved in this method was favorable for aldrin to be converted into dieldrin or that extraction solvents used in this study was not suitable to extract aldrin. The results obtained for spiked and unspiked water samples are summarized in Table 1 and 2.

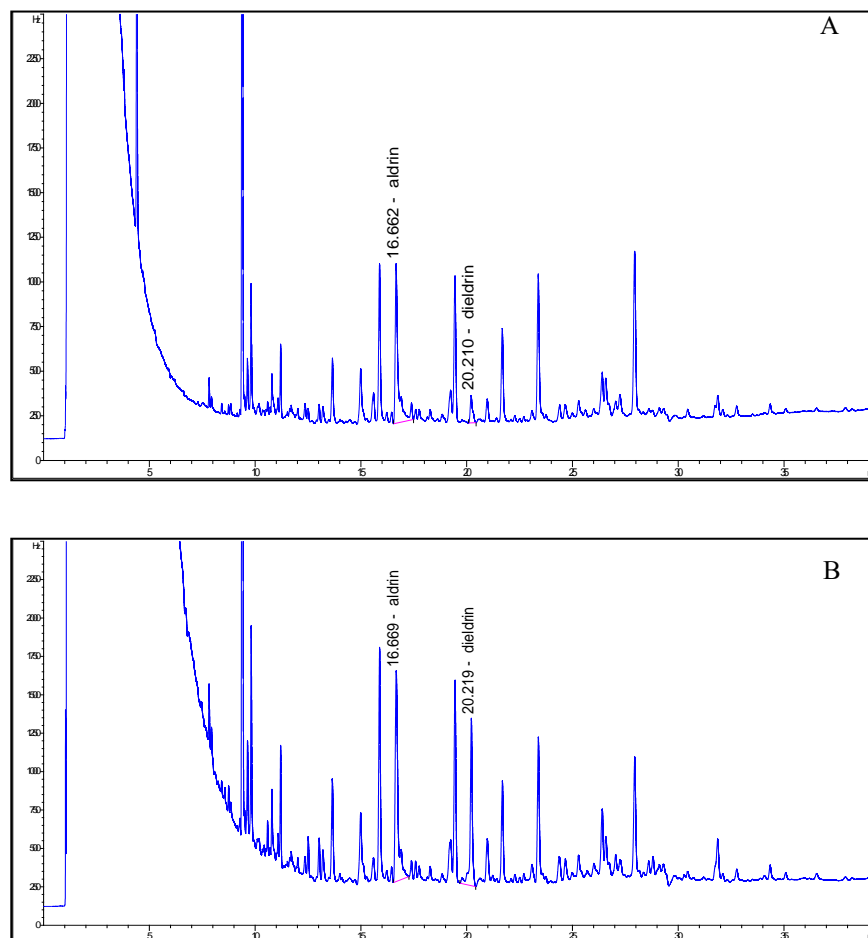


Figure 3 Chromatogram of unspiked water sample (A) and spiked water sample at 0.6 µg/L (B) using optimized LLLE-DLLME method.

LLLE-DLLME was able to pre-concentrate pesticides in river samples at µg/L level. Although the concentration for both pesticides detected is not high (µg/L level), it still exceeded the recommended raw water quality set by Ministry of Health Malaysia and WHO (Guidelines for drinking water quality) for aldrin and dieldrin which is 0.03 µg/L.

Table 1 Unspiked water samples

OCP studied	Conc. found (µg/L)	Conc. factor	Actual conc. after correction (µg/L)	RSD (%)
Aldrin	25.58	94	0.27	15.9
Dieldrin	8.44	94	0.089	9.8

Table 2 Spiked water samples

OCP studied	Spiked conc. (µg/L)	Conc. found after spiking	Conc. factor	Actual conc. after correction (µg/L)	Percentage Recovery (%)
Aldrin	0.6	47.60	94	0.51	39
Dieldrin	0.6	51.47	94	0.55	76

4. CONCLUSION

In this study, a new sample preconcentration technique named LLE-DLLME coupled with GC-µECD for detecting aldrin and dieldrin in river water sample was investigated. The extraction method studied offers some attributes such as simple to perform with no specific equipment required, fast extraction time, lesser amount of solvents and relatively inexpensive. The method also allows extraction without the need to filter the water sample prior to analysis, therefore analytes that may be present in the suspended solids can be extracted as well. Some important parameters affecting the formation of the three phases system and efficiency of extraction were successfully investigated and optimized. Lastly, the optimized conditions for LLE-DLLME were successfully applied on the analysis of river water of Sungai Skudai for the presence of aldrin and dieldrin. Results showed that residual of both pesticides studied were present and the concentrations level for both pesticides exceeded the limit set by WHO and Ministry of Health Malaysia for drinking water quality standards. However, the recoveries obtained were low. This method is still in the development process and more works need to be conducted to be accepted as a new extraction method. However, the advantage of eliminating one pre-filtration step of the suspended solids should be encouraging.

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