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Determination of Dichlorodiphenyltrichloroethane from Soil Using Solid-liquid Extraction and Gas Chromatography–Electron Capture Detector

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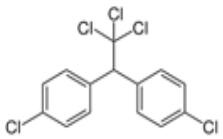
GRAPHICAL ABSTRACT



Structure of DDT

ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) pesticide has been banned around the world due to their adverse effect towards human health and environment. Most of the method development for the analysis of DDT is based on water matrices. Therefore, in this study, soil has been proposed as an alternative matrix in this study. The solid-liquid extraction (SLE) method was utilized to extract DDT from soil and analysis using optimized conditions of gas chromatography and electron capture detector (GC-ECD). N-hexane: deionized water containing 10 % NaCl with solvent ratio 1:9 was used in this study. The optimized method was validated by evaluating calibration curve, linearity, limit of detection (LOD), limit of quantification (LOQ), percentage recovery and intra-assay and inter-assay precision accuracy. From the calibration curve (0.20-5.00 ppm), good coefficient of determination was obtained at R^2 = 0.9980. Limit of detection (LOD) and limit of quantification (LOQ) were obtained at 0.23 ppm and 0.78 ppm respectively. From intra and inter-assay lower coefficient variance (intra: 2.22 - 5.37 %; inter: 0.19 - 2.03 %) and lower percentage error were attained (intra: 7.36 - 8.68 %; inter: 5.66 - 9.89 %). A mean recovery of 93.24 % with good coefficient of variance (CV = 7.51 %) were also acquired. However, from the stability study (30 days) the decreasing pattern of recovery was obtained. The study showed that the analysis of DDT in soil is robust, hence it can be used as an alternative matrix for this analysis.

Keywords: Dichlorodiphenyltrichloroethane, solid-liquid extraction, gas chromatography and electron capture detector, pesticide

1. INTRODUCTION

The presence of pesticides will help to improve the quality, quantity and diversity of food supply in agricultural producers. Besides, it also can control pests and plant disease vectors which may improve livestock yields and livestock quality [1]. Controlling human disease vectors and nuisance organisms which can saved human and animal lives also one of its benefits. There are a few types of pesticides which are classified based on their target pest group for example, insecticides, fungicides, and herbicides. It also can be grouped into chemical families which include organochlorines, organophosphates, and carbamates [2]. The division type of pesticides is based on the target pest group. Organochlorine compounds are classified as insecticides, as its most relevant role is in the control of insect pests. It is a chlorinated hydrocarbon that has been widely used from the 1940s to the 1960s [3] in agriculture and mosquito control due to low prices and high effectiveness.

Organochlorine pesticides have a stable chemical properties and high risk to the environment and human health. The example of organochlorine pesticides group is dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). The DDT cumulative world production was previously estimated at 2,000,000 tons [4]. However, due to its physical properties, high hydrophobicity, biomagnification, persistence, and potential health risks [5], it was banned in most countries in the early 1980s. Moreover, since the properties of organochlorine pesticides is highly insoluble in water and cause them to attach with the soil strongly.

DDT is one of the examples of organochlorine pesticides with chemical structure shows in Figure 1. DDT is a white crystalline solid with no scent or taste [6]. DDT has a molecular formula $C_{14}H_9Cl_5$ and molecular weight 354.49 g/mol with boiling point 260 ^oC. It is made by the reaction between chlorine gas and the double benzene ring structure under optimal temperature and pressure conditions. When both compounds react, DDT is easily formed due to the high reactivity of chlorine gas. Furthermore, DDT has low solubility in water and high solubility in fats thus it will give a non-polar molecule due to its insolubility in water and because of its molecular shape.

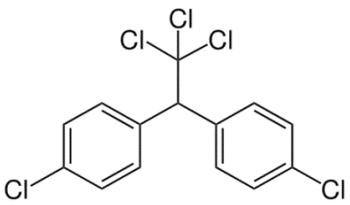


Figure 1. Structure of DDT

2. EXPERIMENTAL

This study was based analysis of DDT from soil which extraction method performed via SLE and GC-ECD analysis was chosen as the instrumentation for qualitative and quantitative analyses. Analysis of DDT from soil was performed via SLE method with 4-bromonitrobenzene as internal standard. The extraction method was modified from previous researchers. Standard of DDT was obtained from Supelco (U.S.A), 4-bromonitrobenzene from TCI (Tokyo) as internal standard, n-hexane and anhydrous sodium chloride was provided by Merck (Germany). Anhydrous sodium sulphate was provided by GCE Laboratory Chemicals (Singapore).

2.1. Preparation of standard and spiked sample.

All stock solutions and working solutions were prepared using n-hexane and stored at refrigerator prior to analysis. Lower concentration of stock solutions was prepared from the 1000 ppm standard. Serial dilution of DDT standards (0.5 to 5 ppm) was then prepared using n-hexane with addition of 4-bromonitrobenzene (5 ppm) as internal standard. For spiked soil sample, 50 g of soil was air-dried at about 40 ^{o}C and then sieved through a mesh with grain size 2 mm. Then, the samples were stored at room temperature. Meanwhile spiked samples were prepared by adding 100 µL of 10 ppm DDT for each 1 g of soil. Then, the samples were proceeded to extract by solid-liquid extraction.

2.2. Solid-liquid extraction

1 mL of n-hexane was added to 1 g of soil in a falcon tube and mixing for 3 minutes by vortex mixer and sonicate for 5 minutes. For percentage recovery analysis the matrices were added with 100 µL of 10 ppm DDT. Then, 9 mL of deionized water containing 10 % NaCl was added and vortex for 3 minutes before further sonicate for 5 minutes. The organic phase layer was then extracted into another tube containing 0.1 g anhydrous sodium sulphate (Na_2SO_4). The remaining solution in the falcon tube was added with 1 mL n-hexane and vortex for 3 minutes. Again, the organic phase layer was extract and combined with the previous extracted organic phase layer. Finally, internal standard was added and preconcentrate under nitrogen evaporator before the extract was mark up until 1 mL in volume.

2.3. Gas chromatography-electron capture detector

A gas chromatography 7890B coupled with electron capture detector (GC-ECD) from Agilent technologies was used in this study. Helium gas was used as a carrier gas in the GC. Agilent technologies HP-5MS capillary column of dimensions (30 m x 320 µm, 0.25 µm film thickness) was used for separation. Initial oven temperature was 200 ^{o}C, initial hold time 1 min, oven ramp 1 rate at 17 ^{o}C min^{-1}, oven ramp 1 rate at 300 ^{o}C, oven ramp 1 hold time at 3 min, and total run time 9.88 min.

3. RESULTS AND DISCUSSION

3.1. Retention time of Standard

Peak of DDT and IS was eluted at retention time 6.277 min and 2.606 min. The efficiency of the column for both DDT and IS was calculated at N = 307264 and N = 17161 respectively which shows high theoretical plate number that can provide efficient separation. Furthermore, the resolution between these two components was found to be $R_S = 90$ where it shows a complete resolution happened in the column and good selectivity due to the excellent separation between these two peaks (Ahuja, 2003). However, it can be found that from IS, the peak showed tailing characteristics. Therefore, the asymmetry factor was then calculated, and the calculated value is $A_S = 1.25$. Since the value $A_S < 2$, it is indicated that the peak is acceptable for the analysis [7]. The optimize results are shown in Figure 2.

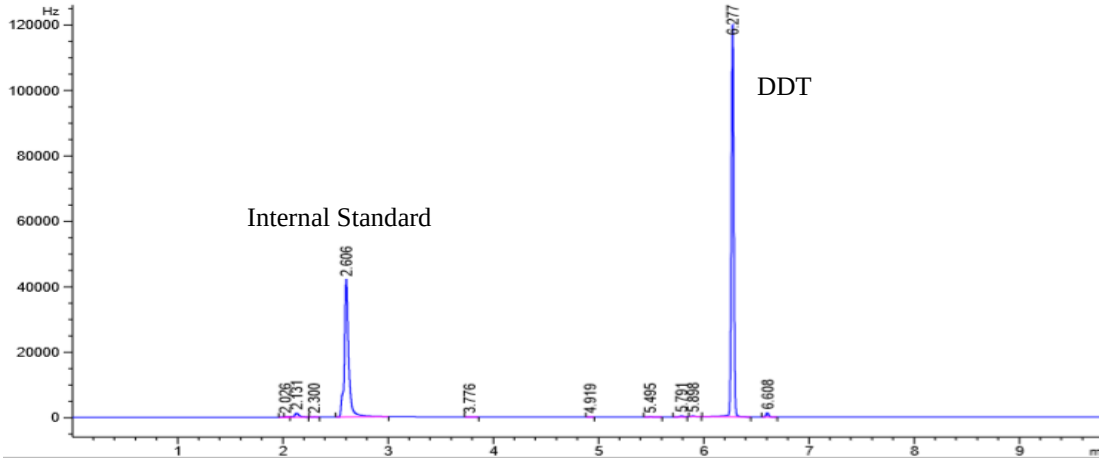


Figure 2. Chromatogram of the separation of DDT and internal standard

3.2. Calibration Curve and Limit of Detection

The peak area response ratio of DDT upon internal standard versus the corresponding calibration concentration (0.20 – 5.00 ppm) of DDT was plotted in the axes as shown in the Figure 3. A good coefficient of determination of R2 = 0.998 was achieved and linear regression equation of y=0.2234x+0.0173 was obtained.

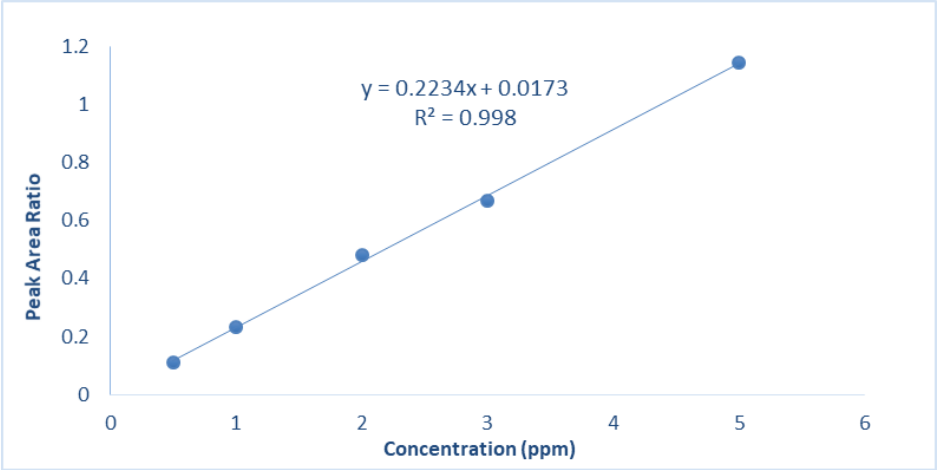


Figure 3. The calibration curve of mean peak area ratio (DDT to IS) against concentration of DDT

The limit of detection (LOD) and limit of quantification (LOQ) is determined from the calibration curve. From the calibration curve the LOD and LOQ of the study were found at 0.23 ppm and 0.78 ppm respectively.

3.3. Intra-day and Inter-day Precision and Accuracy Analysis

The finding of the study shows that the CV % for 1, 3 and 5 ppm of intra-day were 3.73, 2.18 and 5.32 % and the CV % of inter-day were 0.19, 0.50 and 2.03 % respectively which were satisfactory as it below 20 %. Also the study shows that the percentage error for 1, 3 and 5 ppm of intra-day were 7.64, 8.68 and 7.36 % and the error for inter-day were 7.81, 9.89 and 5.66 % which were also fulfil the target criteria. Table 1 and 2 showed the details of assay for intra-day and inter-day respectively.

Table 1. Assay of precision and accuracy of DDT for intra-day

Analyte	Nomial Concentration (ppm)	Intra assay		
		Calculated Concentration ± SD (ppm)	Precision (CV %)	Percentage error (%)
DDT	1	(1.11 ± 0.0416)	3.73	11.46
	3	(3.26 ± 0.0711)	2.18	8.82
	5	(5.34 ± 0.2839)	5.32	6.78

Table 2. Assay of precision and accuracy of DDT for inter-day

Analyte	Nomial Concentration (ppm)	Inter assay		
		Calculated concentration ± SD (ppm)	Precision (CV%)	Percentage error (%)
DDT	1	(1.08 ± 0.0020)	0.19	7.81
	3	(3.30 ± 0.0164)	0.50	9.89
	5	(5.28 ± 0.1074)	2.03	5.66

3.4. Recovery Percentage

Three ratio (1:9, 2:8, 3:7) of n-hexane: deionized water containing 10 % NaCl was done to determine the beast recovery percentage. From the findings (Table 3 and Figure 4), it was observed that method of using 1:9 ratio gave the best percentage recovery as compared to other ratio with 89.13% recovery. Then it follows with ratio 2:8 and 3:7 at 87.34 % and 83.25 % respectively. From the study done by Al Mahmud et al (2015) [8], he succeed to obtain recovery for the analysis of pesticides in soil matrix from range of 72 to 120 %. Therefore this analysis was proceed with 1:9 ratio for the stability study of analyte in soil matrix.

Table 3. Percentage recovery of solvent at different ratio

Solvent Ratio (hexane:water)	Estimated Concentration (ppm)	Calculated concentration ± SD (ppm)	CV (%)	Recovery (%)
1:9	1	0.80 ± 7.11	7.98	89.13
2:8	1	0.75 ± 10.02	11.48	87.34
3:7	1	0.77 ± 5.87	7.05	83.25

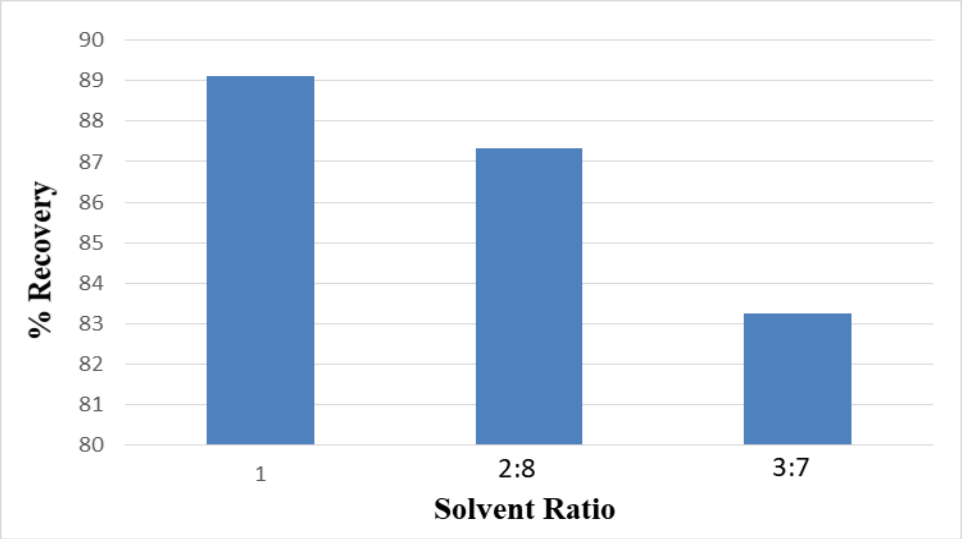


Figure 4. Percentage recovery comparison of different solvent ratio

3.5. Stability of DDT in soil

Stability of DDT was studied for 30 days and extracted using solvent ratio of n-hexane: deionized water containing 10% NaCl (1:9). The percentage recovery for the stability of DDT in soil for 30 consecutive days is tabulated in Table 4 and is illustrated in Figure 5.

Table 4. The percentage recovery of consecutive days

Days	Recovery (%)
Day 1	55.46
Day 5	30.92
Day 10	22.99
Day 15	15.88
Day 25	13.59
Day 30	17.64

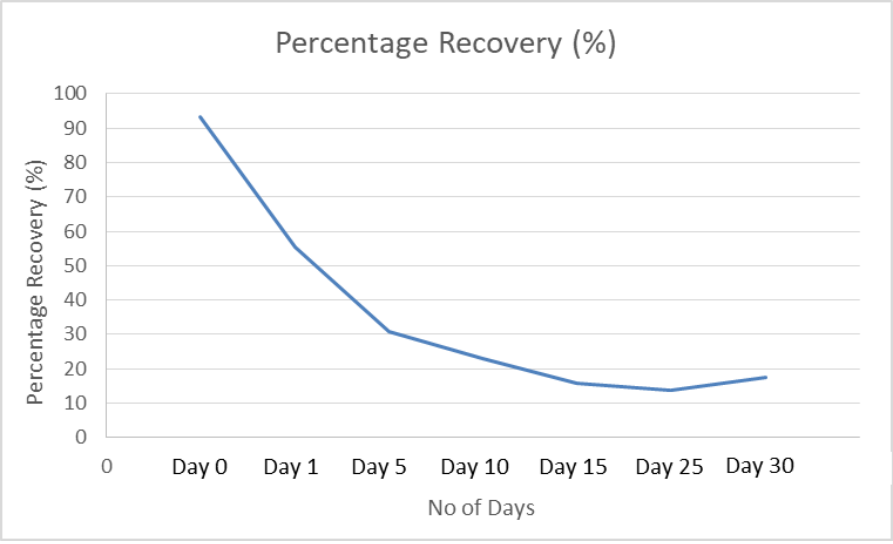


Figure 5. The stability of DDT in the consecutive days

For Day 0 the extraction was done an hour after spiking the standard into the soil sample. The next extraction of Day 1 was executed after 24 hours. From Day 0 to Day 1, the pattern of the curve shows steep decreasing pattern (93.24 % to 55.46 %) before showing a stable decreasing pattern up to day 30. The decreasing pattern was also observed by Smith and Parr, 1972. This may be due to rapid dehydrochlorination of DDT which may not detectable by an electron capture principle because of reduced electron sensitivity [9].

3.6. Soil Sample Analysis around Skudai Area

Soil samples were collected at a few location in Skudai area. Soil samples from different nursery namely Chaqura Garden, Rimbun Ventures, Lucky Flowering, Nature at Work, and UTM's farm were taken for this study. Triplicate soil samples were prepared from each sample locations and extracted using optimized method of extraction with addition of internal standard prior to chromatographic analysis. The chromatograms of the SLE extracts from each nursery are shown in Figure 6. From the chromatogram, there were no DDT peaks could be observed.

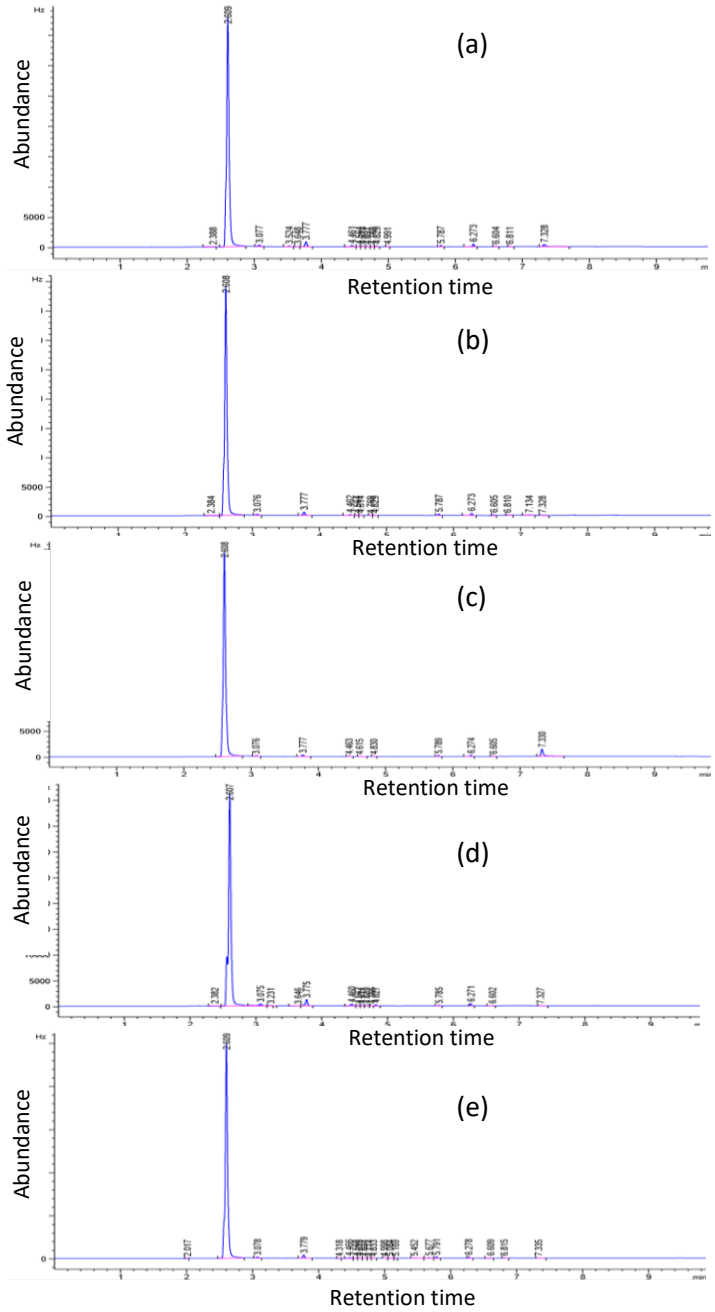


Figure 6. GC-ECD chromatogram of soil sample from (a) Chaqura Garden, (b) Rimbun Ventures, (c) Lucky Flowering, (d) Nature At Work and (e) UTM's farm

4. CONCLUSION

The study found that the analysis of DDT from soil using GC-ECD could be done using solid-liquid extraction. The optimized extraction method employed in the study was cost effective and also gave good percentage recovery which is more than 80 %. Short period of time was needed for sample preparation as well as instrumentation analysis. From the calibration curve obtained in the study, excellent coefficient of determination (R^2 = 0.9980). Lower limit of detection (LOD = 0.23 ppm) as well as lower limit of quantification (LOQ = 0.78) were also acquired in this study. From the precision and accuracy analysis of intra and inter-day, lower CV % (intra: 3.92, 2.22 and 5.37 %; inter: 0.19, 0.5 and 2.03 %) and lower percentage error were attained (intra: 7.64, 8.68 and 7.36 %; inter: 7.81, 9.89 and 5.66 %). For the extraction of DDT from soil, the optimized method (n-hexane: deionized water containing 10 % NaCl; 1:9) showed good percentage recovery (93.24 %) with low coefficient variance (7.51 %). For the stability studies, rapid dehydrochlorination were observed from Day 1 to Day 30 .This may be either due to lost in the aqueous phase during extraction, or not detectable by an electron capture principle because of reduced electron sensitivity. In overall, the finding of the study proved the availability of soil as one of the matrix that can be used for environmental forensic analysis. This is because the soil is collected directly from the location of agriculture would provide accurate analysis as well as pinpointing the user of illegal the pesticide. The optimized method is also cost effective, rapid and efficient.

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