NanoPak-C All Carbon solid phase extraction (SPE) of Organochlorine Pesticide from Trace Amounts of Environmental Samples

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Abstract

Introduction: Organochlorine pesticides (OCPs) are synthetic chlorinated hydrocarbon derivatives widely used in global agriculture. Due to their toxicity, OCPs pose human health and environmental concerns. Complex matrices hinder the detection and quantification of OCPs with liquid chromatography methods (column fouling and baseline noise). Thus, solid-phase Extraction (SPE) is a standard sample preparation method to clean up environmental samples and isolate OCPs before their introduction into chromatography systems. There is a shortage of SPE techniques to extract OCPs from small quantities of environmental samples. We present an optimized method to prepare samples using solid phase extraction to detect 15 OCPs in small amounts (100 mg soil and 200 µL river water) of environmental samples.

Methods: OCP spiked River water and Soil samples were prepared and sent through SPE cartridges. Cartridge volume and bed weight variations were tested (1, 3, 6 mL at 30, 50, 100, 250, 500 mg). Recovered samples were evaluated with GCMS.

Results: From both water and soil samples, all OCPs were recovered across all tested bed weights and cartridge volumes of the novel carbon microbead sorbent. Average pesticide recovery from river water is reported from 68.79 ± 8.01 through 275.15 ± 67.61 (µg/kg) across the six tested bed weights. Recovery from soil samples ranges from 117.15 ± 42.52 through 158.85 ± 101.5 (µg/kg) across the six tested bed weights.

Conclusion: All-carbon reversed-phase SPE media at bed weights between 30-500 mg provide recovery of OCPs from small quantities of soil and water samples. The streamlined SPE procedure is suitable for extracting OCPs from environmental samples.

Introduction

Organochlorine pesticides (OCPs) are synthetic chlorinated hydrocarbon derivatives widely used in global agriculture. Due to their toxicity, OCPs pose concerns for human health and environmental stability. To assess ecotoxicological risk, river water, agricultural soil, and sediment are routinely monitored for OCPs.[1,2]

Complex matrices in environmental samples hinder the detection and quantification of OCPs using chromatography methods directly (column fouling and baseline noise).[3] Thus, solid-phase extraction (SPE) is used as a sample preparation method to clean up environmental samples before they are introduced into chromatography systems. Currently, the literature offers limited SPE techniques for extracting OCPs from environmental samples in small quantities. Using novel all-carbon reversed-phase media packed in SPE columns, this study focuses on developing reliable methods for extracting and detecting OCPs from small samples of soil (100 mg) and river water (200 µL).

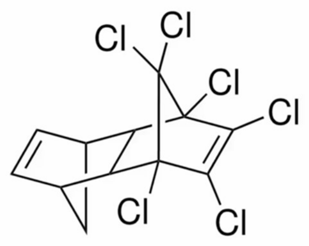


Figure 1: Representative Organochlorine pesticides (Aldrin) routinely used in agricultural treatments that bioaccumulate aquatic organisms.

This white paper presents a simple preparation method for extracting OCPs from small quantities of complex environmental samples using our NanoPak-C all-carbon microbead media. We demonstrate an optimized solid-phase extraction (SPE) protocol for isolating and detecting 15 key organochlorine pesticides (OCPs) in small quantities (100 mg soil and 200 µL river water) of environmental samples.

Materials and Methods

Chemicals

HPLC-grade Isooctane (99%) and N-Hexane (99%) were purchased from Thermo Fisher Scientific (India). Dichloromethane (99%) sourced from Merck Life Science (USA) and Methanol (99%) from Rankem were HPLC grade and used as received. EPA Pesticide Mix product No. 48858-U (Supelco Merck, USA) were the probe analytes in this study, namely: ALPHA-BHC, BETA-BHC, GAMMA-BHC, DELTA-BHC, HEPTACHLOR, ALDRIN, HEPTACHLOR EPOXIDE ISOMER B, ENDOSULFAN I (ALPHA), 4,4'-DDE, DIELDRIN, ENDRIN, 4,4'-DDD, ENDOSULFAN II (BETA), 4,4'-DDT, ENDOSULFAN SULFATE. The pesticide mix was stored at 4°C and used to prepare working standards in deionized water. A Milli-Q water purification system (LabLink, India) provided the deionized water. PTFE syringe filters (0.45 µm) were used for sample filtration.

SPE columns and Manifold

8 SPE variations employing different cartridge volumes and bed weights were used in this study (1ml 30mg, 1ml 100mg, 3ml 30 mg, 3ml 50mg, 3ml 100mg, 3ml 250mg, 3ml 500mg, 6ml 500mg). NanoPak-C graphite microbeads were manually packed into empty straight barrel SPE cartridges at the prescribed bed weights between a lower and upper frit. A 24-port vacuum manifold was used for the extraction process.

OCPs stock preparation

80 µL of EPA Pesticide Mix was diluted in 800 µL of di water to prepare a stock solution for use.

Sample Preparation, SPE Extraction, GC-MS/MS Analysis

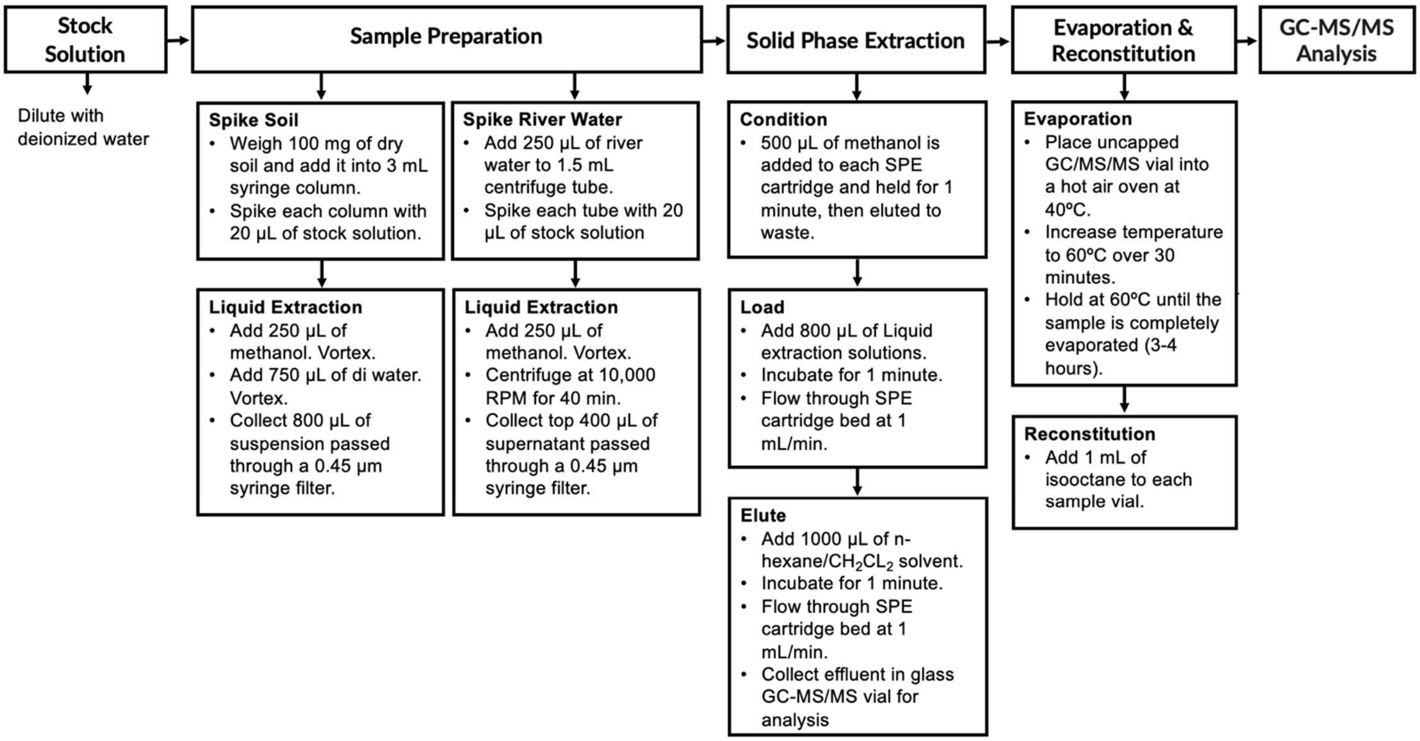
Soil was gathered from a lot of pristine land located in a military base that was not used for agricultural production in the Nashik region of Maharashtra, India. 100 mg dry soil samples were transferred to 3 mL syringe columns. Each column was spiked with 20 µL of pesticide stock solution. Next, 250 µL of methanol and 750 µL of deionized water were added to the sample, thoroughly shaken, and passed through a 0.45 µm syringe filter into a waiting RIA vial.

River water was directly sampled from the Godavari River, Nashik region of Maharashtra, India.
Before use, water samples were carefully centrifuged to remove larger contaminants. 250 µL river water samples were spiked with 20 µL pesticide stock solution. 250 µL of methanol was mixed into the sample prior to centrifugation at 10,000 rpm for 40 minutes. 500 µL of the resulting supernatant was passed through a 0.45

µm syringe filter into a waiting RIA vial. An additional 500 µL of deionized water was added to each sample and mixed.

Solid Phase Extraction (SPE). Each SPE cartridge was preconditioned with 500 µL of methanol. 1000 µL samples were passed through the cartridges at a 1 mL/min flow rate controlled with a vacuum manifold. The analytes retained on the cartridge were eluted using 1 mL of N-hexane: DCM (70:30). The eluent was dried in an oven at 60°C. Finally, the residue was reconstituted with 1 mL of Iso-octane and sent for GC-MS/MS analysis.

GC-MS/MS Analysis. An Agilent GC-7890B, MS-7000D GC-Mass spectrometry system, fitted with DB-5ms Ultra Inert (5m • 250 µm • 0.25 µm) columns were used for analysis. 2 µL samples were injected using helium as carrier gas (2.25 mL/min) and nitrogen as CID gas (1.50 mL/min), at 280°C using split-less injection mode. The MS/MS run mode was electron ionization (EI), 70eV, scanning range 10 – 500 amu, source and transfer line temperature at 280°C with MS1 and MS2 Quad at 150°C.



Method Flowchart

Results and Discussion

All OCPs were recovered from soil and river water samples using SPE cartridges packed with the NanoPak-C All-Carbon Microbeads, as shown in **Figures 2A and 2B**. Each cartridge volume at each bed weight bound and eluted back each OCP when a suitable solvent was applied. Recovery for each unique pesticide as measured by GCMS (µg/Kg) positively correlated with the spiking concentration of the pesticides for both river water and soil samples. Average pesticide recovery from river water is reported from 68.79 ± 8.01 through 275.15 ± 67.61 (µg/kg) across the six tested bed weights. Recovery from soil samples ranges from 117.15 ± 42.52 through 158.85 ± 101.5 (µg/kg) across the six tested bed weights.

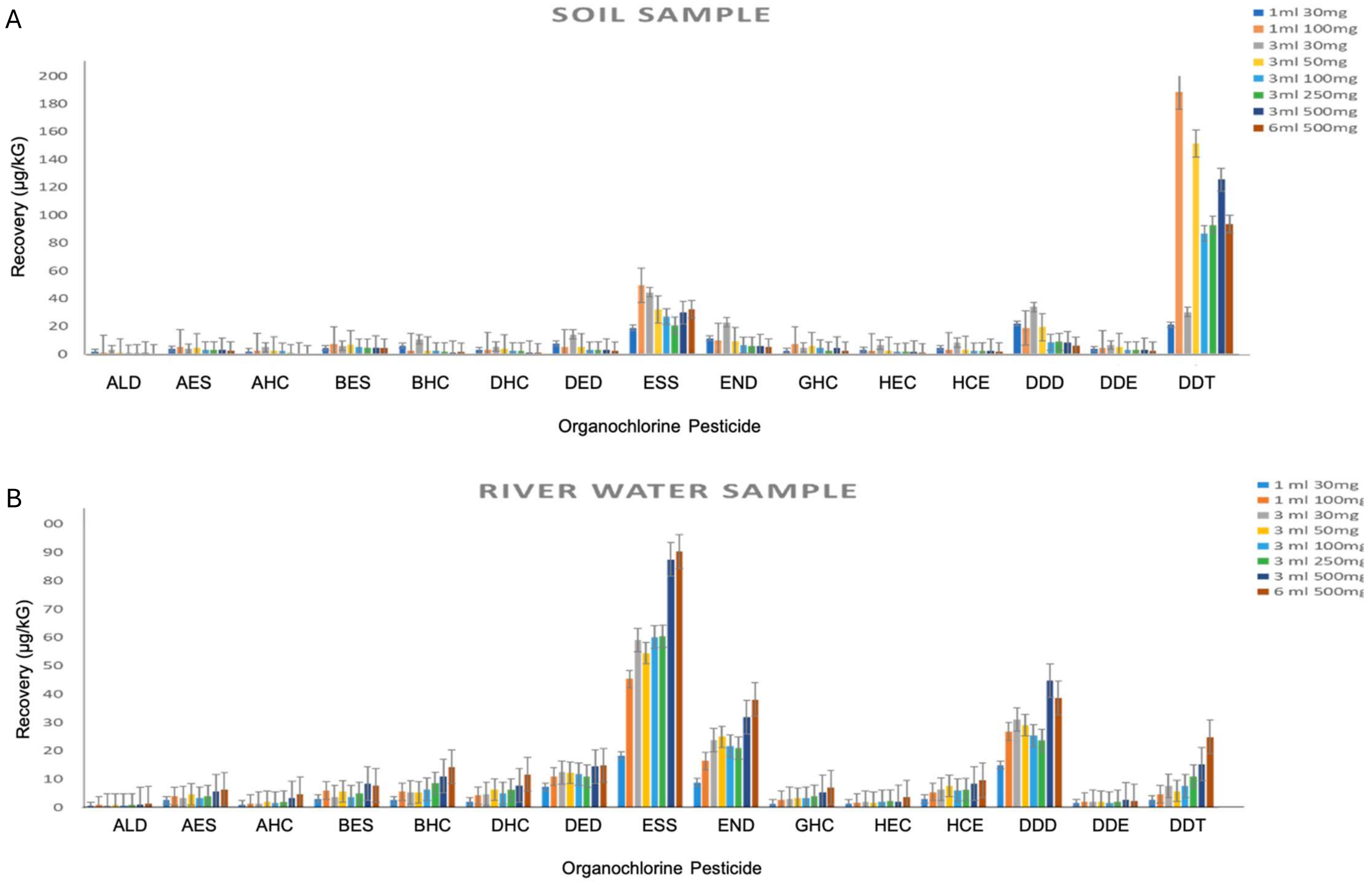


Figure 2: All organochlorine pesticides are recovered across all bed weights and cartridge volumes. OCP Solid Phase Extraction on 1 mL, 3 mL, 6 mL cartridges packed with NanoPak-C All-Carbon Microbeads at 30mg, 50mg, 100mg, 250mg, 500mg bed weights. (A) Recovery from 100mg soil samples. (B) Recovery from 250 µL river water samples. OCPs are identified as follows: ALD = Aldrin, AES = alpha endosulfan, AHC = alpha BHC, BES = beta endosulfan, BHC = beta BHC, DHC = delta BHC, DED = dieldrin, ESS = endosulfan sulphate, END = endrin, GHC = gamma BHC, HEC = heptachlor, HCE = heptachlor epoxide, DDD = p,p-DDD, DDE = p,p-DDE, DDT = p,p-DDT.

No significant differences in pesticide recovery were observed between SPE cartridge volume (when controlled for bed weight) and bed weight (when controlled for cartridge volume). Looking more closely at the river water data set, matched for equal bed weight (100 mg), 1 mL and 3 mL SPE cartridges produced OCP recovery values of 9.05 ± 0.87 and 10.54 ± 1.70 respectively, for which the difference was not statistically significant p = 0.17. Matched for equal cartridge volume (3 mL), 30, 50, 100, 250, and 500 mg bed weight SPE cartridges produced OCP recovery values of 11.03 ± 2.43, 11.02 ± 6.36, 10.54 ± 1.70, 11.15 ± 1.42, 16.65 ± 3.96 respectively, again for which the differences were not statistically significant p = 0.15 (**Figure 3)**.

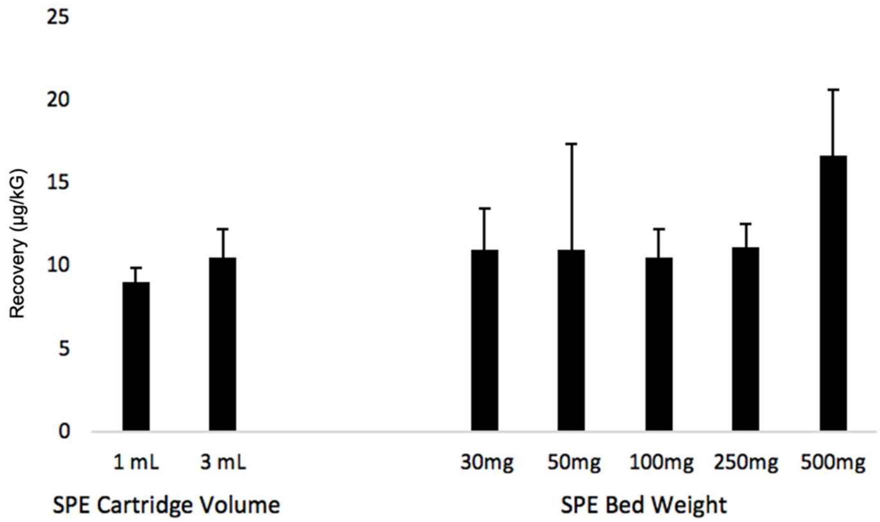


Figure 3: No difference observed in average OCP recovery between bed weights or cartridge volumes. Matched for equal bed weight (100 mg), 1 mL and 3 mL experimental SPE cartridges did not show significant differences in OCP recovery ($p = 0.17$). Matched for equal cartridge volume (3 mL), experimental SPE cartridges did not show significant differences in OCP recovery ($p = 0.15$).

Conclusion: NanoPak-C All-Carbon reversed-phase SPE media at bed weights between 30-500 mg recover organochlorine pesticides from small quantities of environmental samples with complex matrix compositions. This streamlined SPE procedure is suitable for extracting OCPs from soil or water samples available in limited or large quantities. The methods described demonstrate that this sample preparation technique is cost-effective and easy to use. Despite the complex nature of the soil and water samples studied, robust recovery for all targeted pesticides exhibits Nanopak-C All-Carbon Microbeads as a promising SPE reversed-phase media for field testing of environmental samples. This method is suitable for routine monitoring, confirming, and quantifying trace OCP levels in agricultural soil and water resources.

References:

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