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DETERMINATION OF PENTACHLOROPHENOL IN WATER AND AQUIFER SEDIMENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Methods for the determination of pentachlorophenol (PCP) in water and aquifer sediments are presented. Reverse-phase high-performance liquid chromotography employing ion suppression and gradient elution is used. PCP can be determined directly in water at a lower limit of detection Of 0.2 micrograms per liter. For extracts of sediment, PCP can be determined to a lower limit of 1.0 micrograms per kilogram.

INTRODUCTION

Pentachlorophenol (PCP) is used quite extensively as a herbicide, fungicide and insecticide. It is mainly used for the preservation of wood and wood products. PCP, second to creosote, is the most widely used pesticide in the United States, having an annual production of over 80 million pounds (Cirelli, 1978). As a result of wide usage, PCP has been found in river waters, water supplies and sewage effluents (Buhler et al., 1973). Starr, 1969, and Pierce and Victor, 1977, showed the PCP was accumulated in tissue from fish taken from surface water contaminated by industrial discharge of the chemical. Importantly, PCP is a highly toxic compounds (Bevenue and Beckman, 1967) and is specified by the United States Environmental Protection Agency as a priority pollutant.

Gas chromatography is the method most often used for the determination of PCP in environmental samples (Kuehl and Dougherty, 1980, and Boyle et al., 1980). Gas chromatographic procedures require solvent extraction and derivitization to improve volatility of PCP. These two steps of the analysis are undesireable because they contribute to error and expose the analyst to solvents and organic reagents.

Ervin and McGinnis, 1980, demonstrated the PCP could be determined by normal phase high performace liquid chromtography (HPLC). They were able to analyze solvent extracts of wood, wood treating fluids and

waste waters for PCP without derivitization. Hayes, 1979, used reverse phase HPLC in conjunction with a mobile phase composed of ion-pairing reagent, tetraammonium phosphate dissolved in methanol-water solution. The ion-pairing reagent improved the retention characteristics of PCP in the reverse phase system thus allowing direct analysis of aqueous formulations.

Although the ion-pairing technique eliminated the solvent extraction step, this procedure does not permit the detection of low levels of PCP as expected in most environmental samples. In this laboratory, the necessary sensitivity was achieved by employing ion suppression and gradient elution in the reverse-phase system and is the method presented in this report.

METHOD

1. Summary of method

PCP can be determined directly in water, microbial growth media, and aqueous extracts of sediments. Following centrifugation, the sample is filtered and acidified preparatory for analysis. PCP is determined by HPLC using a reverse-phase column and an ultraviolet detector. The lower limit of detection is 2×10^{-9} g. The usual injection volume is 2.0 ml which permits a lower detection limit of $1\ 0 \times 10^{-6}$ g/L in the sample. If desired, it is practical to inject 10.0 ml of sample and lower the detection limit to 0.2×10^{-6} g/l.

2. Interference

Any compounds having chemical and physical properties similar to PCP may cause interference. Ion supressed PCP is retained longer on the chromatographic column than all the isomers of chlorophenols including 2, 3, 4, 6 - tetrachlorphenol, the major impurity in commercial PCP formulations. Napthol, phenol, and selected methyl phenols elute before PCP and do not interfere. The phenoxy acid herbicides commonly used in conjunction with PCP are well separated from the subject compound. Since PCP is mainly used as a wood preservative, the association with creosote must be suspected. The methyl naphthalene components of creosote interfere

and must be removed, if present.

3. Apparatus

3.1 Centrifuge: Of bench-top type, accomodating 15-ml centrifuge tubes and capable of speeds to 3000 revolutions per min. (rpm).

3.2 Chromatograph: A high-performance liquid chromotograph capable of operating to pressures of 4000 lb/in^2, having an injection port and an ultra-violet detector sensitive at 254 nm (nanometers). A Water Associates ALC/GPC 204 liquid chromatograph equipped with the dual-channel detector, a model-6000A solvent delivery system, and a model-660 solvent flow programmer was used.

3.3 High-performance liquid chromatographic column: A stainless steel column 300 mm long with a 3.9 mm inside diameter, packed with reverse-phase material having a particle size of 10 um (micrometer). A Waters Associates μBondapak C_{18}, part number 27324, was used

3.4 Filtration apparatus: All-glass filter apparatus for vacuum filtering, using 47-mm diameter membrane filters with a funnel volume of 300 mL and a flask volume of 1 L. Millipore part no. xx 15 047 00, was used. A Swinny-syringe filtration apparatus consisting of a 10-mL glass syringe and a 25-mm diameter filter holder having standard Luer fittings. Millipore part no. xx 30 025 00 was used.

3.5 Filter membrane: A membrane inert to acetonitrile, having a pore diameter of 0.45 μm or less, for vacuum filtration. Selas Flotronics silver-membrane filters catalog no. FM-47.45 were used. To filter the aqueous mobile phase Nuclepore Corp. polycarbonates filters stock no. 111107 were used. For syringe filtration of the aqueous samples, Nuclepore Corp. polycarbonate filters stock no. 110607 were used. The polycarbonate filters were rinsed with reagent water prior to use.

3.6 Microbalance: A Cahn Gram Electrobalance or equivalent.

3.7 Recorder-digital integrator: A strip-chart recorder having a chart speed of 0.5 cm/min and a pen speed of 1-sec or less and a digitizing device having a linearity of ± 0.1 percent of reading. A Waters Associates Data Module was used.

3.8 Syringes: Microsyringes having a capacities of 10, 100 and 500 μL, accurate to ± 1.0 percent and 5, 10 and 20 mL syringes ± 1.0 percent were used.

4. Reagents

4.1 Acetic acid, glacial. ACS reagent grade

4.2 Acetonitrile, of quality suitable for high-performance liquid chromatography.

4.3 Aqueous mobile phase: Prepared by adding 2.0 ml acetic acid to 1L of reagent water.

4.4 Hexane, normal, of HPLC quality.

4.5 Hydrochloric acid, ACS reagent grade.

4.6 Pentachlorophenol: Reference grade, having a melting point of 190° C. PCP is obtainable from chemical specialty suppliers.

4.7 Sodium hydroxide, ACS reagent grade.

4.8 Water reagent, distilled, obtained from a high-purity tin-lined still. The feed water is passed through an activated carbon filter. The distillate is collected in a tin-silver storage tank and the stored water is irradiated by ultraviolet light. A gravity delivery system is used and no plastic material other than teflon is in contact with the distilled water. For HPLC, the water is filtered through a 0.4 µm polycarbonate filter.

5. Calibration

The chromatographic system must be calibrated using a PCP reference standard under operating conditions to be used in the analysis.

5.1 Operating conditions: After injection the sample components are eluted from the column using a gradient from 100 percent aqueous mobile phase to 100 percent acetonitrile. The gradient is completed in 10 min and is conducted at a constant flow of 2.0 mL/min

5.2 Calibration standard: A calibration standard is prepared at nearly the same concentration as expected in the sample. For samples at or near minimum detection levels, the system is calibrated using a standard 10-100 times greater than the minimum detectable amount. A 2.00 ml injection of aqueous solution containing PCP standard at 20×10^{-9} g/mL produces a response of 0.005 absorption units at a wave length of 254 nanometers (nm) on the chromatographic system described.

6. Procedure

6.1 Sampling: Samples are collected according to recommended practice for organic analysis (see for example, Goerlitz and Brown, 1972, p. 2-3). To ensure quality, the sample is cooled in ice or refrigerated immediately after collection No preservative is used and the sample is analyzed without delay.

6.2 Direct aqueous procedure: Transfer 15 ml of water sample to a centrifuge tube.

6.2.1 Centrifuge the sample. Usually centrifugation at 2000 rev/min for 5 min is adequate.

6.2.2 Prepare the Swinny-syringe filtration unit and clean by filtering 2 mL methanol followed by 5 mL reagent water. Gently express remaining water from the apparatus using the air in the syringe.

6.2.3 Filter the supernatant, discarding the first 2 mL to aid rinsing. Collect 10.0 mL of filtrate in a clean graduated centrifuge tube.

6.2.4 Adjust the pH greater than 11 (test paper) by dropwise addition of 1N sodium hydroxide to the contents of the centrifuge tube. Add 2 mL n-hexane, stopper and shake the tube vigorously for 1 min. Allow the layers to separate and aspirate the hexane from the tube. Note: This step may be omitted if it is known that creosote is not present in the sample.

6.2.5 Acidify to pH 3 using glacial acetic acid and testing with pH paper. Note the final volume of sample.

6.2.6 Perform HPLC analysis on the prepared sample using the same operating conditions as during calibration.

6.3 Sediment analysis: Weigh 20.0 g of wet sediment or core material into a 250 mL beaker The moisture in the sediment is determined on a separate sub-sample.

6.3.1 Add 20.0 ml of 0.1N NaOH and stir the mixture thoroughly using a glass stirring rod. Allow to stand for 15 min.

6.3.2 Decant the liquid layer into a centrifuge tube and centrifuge at 2000 rev/min for 5 min.

6.3.3 Prepare the Swinny-syringe filtration unit and clean by filtering 2 mL of methanol followed by 5 ml of reagent water. Express any retained water from the apparatus using air in the syringe.

6.3.4 Decant the supernatant into the Swinny-syringe unit and filter. Discard the first 2 mL to aid rinsing. Collect 10.0 mL of filtrate in a clean graduated tube. Caution! Polycarbonate filter membranes are attacked by basic solutions. This step should be performed with little delay.

6.3.5 Add 2 ml n-hexane, stopper and mix the contents of the tube vigorously for 1 min. Allow the layers to separate then aspirate the hexane layer from the tube. Note: This step may be omitted if it is known that creosote is not present in the sample.

6.3.6 Acidify to pH 3 using glacial acetic acid, testing with pH paper, and note the final volume in the tube.

6.3.7 Perform HPLC analysis on the prepared sample using the same chromatographic conditions as for calibration.

7. Determination

The chromatographic system is calibrated with PCP standards. The response, absorbance, is proportional to the concentration of compound eluting from the column. The time elapsed from the introduction of the sample to the point of maximum absorbance of the component is designated as the retention time. For an injection volume of 2.0 mL, PCP elutes in 11.26 ± 0.02 min.

7.1 Direct aqueous analysis: The concentration of PCP in water can be calculated using the following equation: The concentration of PCP

$$(ug/L) = \frac{A}{m} \cdot \frac{Vd}{Vo} \cdot \frac{1000}{Vi}$$

where A = area of component response,
m = slope (unit area per ng),
Vd = acidified volume (mL)
Vo = initial aqueous volume (mL), and
Vi = volume (mL) injected into the chromatograph.

7.2 Sediment analysis: The concentration of PCP can be calculated using the following: Concentration of PCP

$$(ng/_{Kg}) = \frac{A}{m} \cdot \frac{Vd}{Vo} \cdot \frac{1000}{Wd} \left(\frac{20+Wo-Wd}{Vi}\right)$$

where A = area of component response,
m = slope (unit area per ng),
Vo = aqueous extract volume (mL),
Vd = acidified extract volume (mL),
Vi = volume (mL) injected into the chromatograph,
Wo = weight (g) of sediment sample
Wd = dry weight (g) of sediment.

8. Discussion

The liquid chromatographic method described is rapid, convenient, and sensitive for the determination of PCP in environmental samples. Both water and aqueous extracts can be analyzed with little pretreatment. The chromatographic separation is made on a reverse-phase column by a programmed gradient elution starting with a mildly acid aqueous mobile phase and ending with an aprotic organic solvent. The ionization of PCP is suppressed sufficiently so that the compound elutes at 11.26 ± .02 min as an unionized, polar component. Compounds often associated with PCP, such as the phenoxy acids listed in table 1, and the chlorophenols in table 2 elute earlier under these conditions. Interference of most

concern in environmental samples are the alkyl phenols, the tar acids occurring in the wood preservative creosote. The elution times of phenol, naphthol and a number of methyl phenols are given in table 3. Again, these compounds elute well ahead of PCP at this low pH and give no interference.

The elution order can be changed by adjusting the pH of the aqueous mobile phase. By increasing the pH to near neutral (0.2 percent ammonium acetate) PCP comes out in 8.35 min, 3 minutes earlier, but elution times for phenol and the methyl phenols are unchanged. The strong phenoxy acid 2, 4-D elutes at 6.90 min or about 2 minutes earlier. The pKa of PCP is 5 whereas phenol and the methyl phenols have pKa's aroung 10 and 2, 4-D has a pKa of 3. By knowing the pKa, one can predict the elution behavior of a compound with respect to PCP.

Adjusting the pH is very useful for fixed mobile-phase composition or isocratic chromatography. For example, mixtures of phenol and PCP in samples taken from a microbial reactor were readily analyzed in less than 10 min. The eluant composition was 35 parts acetonitrile and 65 parts aqueous 0.2% ammonium acetate solution. Phenol eluted in 3.50 min and PCP in 4.40 min.

Some other compounds which may cause interference with PCP are naphthalene and the methyl naphthalenes, components from the neutral fraction of creosote. Fortunately, these compounds are removed with a hexane extraction.

9. Precision

Precision for the direct procedure at 100 μg/L is ± 5 percent. Tests performed using the extraction procedure on sediment samples fortified with 100 μg/Kg gave 90 ± 15 percent recovery.

TABLE 1

Retention times of phenoxy acid herbicides

Compound	Retention times, min.
2,4-dichlorophenoxyacetic acid (2,4-D)	9.07
2,4,5-trichlorophenoxyacetic acid (2,4,5-T)	9.55
2-(2,4,5)-trichlorophenoxy) propionic acid (silvex)	10.05

TABLE 2

Retention times of chlorophenols

Compound	Retention time, min
0-chlorophenol	8.69
p-chlorophenol	8.85
m-chlorophenol	8.96
2,3-dichlorophenol	9.43
2,5-dichlorophenol	9.59
3,4-dichlorophenol	9.59
2,4-dichlorophenol	9.60
2,6-dichlorophenol	9.65
3,5-dichlorophenol	9.89
2,3,6-trichlorophenol	10.05
2,3,4-trichlorophenol	10.08
2,4,6-trichlorophenol	10.22
2,4,5-trichlorophenol	10.25
2,3,5-trichlorophenol	10.31
3,4,5-trichlorophenol	10.32
2,3,4,5-tetrachlorophenol	10.73
2,3,5,6-tetrachlorophenol	10.75
pentachlorophenol	11.26

TABLE 3

Retention times of phenols

Compound	Retention times, min
phenol	7 63
o-cresol	8.54
m-cresol	8.45
p-cresol	8.44
3,4-dimethylphenol	8.94
3,5-dimethylphenol	9.00
2,3-dimethylphenol	9.15
2,5-dimethylphenol	9.19
2,6-dimethylphenol	9.26
1-naphthol	9.46
2,3,5-trimethylphenol	9.63
2,3,6-trimethylphenol	9.70

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