CHROM. 24 246

In situ extraction and derivatization of pentachlorophenol and related compounds from soils using a supercritical fluid extraction system

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ABSTRACT

An *in situ* supercritical fluid extraction and derivatization procedure for the determination of pentachlorophenol (PCP) and related compounds from soil samples is described. Phenols are extracted from soil and acetylated *in situ* with supercritical carbon dioxide in the presence of triethylamine and acetic anhydride at a temperature of 80°C. Quantitative recovery of di-, tri-, tetra- and penta-chlorophenols was obtained by a 10-min extraction with carbon dioxide at 37.2 MPa (365 bar, 0.8 g/ml density) from soil samples fortified to 0.5 and 5 μg/g levels. In a comparison study, the supercritical fluid extracton and the steam distillation methods both produced very similar results for pentachlorophenol and other chlorophenols in a reference sample. When this method is applied to contaminated soils samples collected in a wood treatment plant, results for chlorophenols in a sample can be obtained in approximately 90 min.

INTRODUCTION

Abnormal discoloration of wood, commonly referred to as sapstain, is caused by fungi which derive nourishment from wood cells. Other than by kiln-drying, sapstain and mold on the surface of lumber can be prevented by treatment of wood with anti-sapstain chemicals. Due to their effectiveness, penta-chlorophenol (PCP) and its derivatives have been the most widely used anti-sapstain chemicals in Western Canada over the last 50 years. Recently, the application of PCP by the sawmilling and forestry industries has become an environmental concern

since PCP is toxic to fish and mammals and technical grades of PCP are known to contain the highly toxic chlorinated dibenzo-*p*-dioxins and furans. In response to these concerns, the use of PCP as a wood preservative in British Columbia has mostly been phased out. However, this chemical is still being used in wood-treatment plants in other parts of Canada for special applications.

PCP in soils or sediments can be traditionally determined by solvent extraction techniques (*e.g.* Soxhlet) [1] or by a steam distillation approach [2,3]. In both cases, the extraction process takes a few hours or longer. In the case of solvent extraction, a large amount of solvent must be used and a great deal of coextractives are produced. The latter often create a problem in the subsequent cleanup and chromatographic analysis. If the extracted PCP is to be analyzed by gas chromatography in the form of

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an acetyl, methyl or pentafluorobenzyl derivative, extra time is required for the additional derivatization step.

Supercritical fluid extraction (SFE) has been proven to be a more efficient alternative than existing solvent extraction techniques for most solid samples. It has been successfully applied to the determination of polychlorinated biphenyls [4], chlorobenzenes [5], polycyclic aromatic hydrocarbons [4,6], dioxins [7], resin and fatty acids [8] in sediment and other matrices and the list is growing rapidly. Because of the non-polar nature of supercritical carbon dioxide, extraction recovery of polar parameters is low unless a modifier such as methanol is added to the system. SFE of free pentachlorophenol from a soil sample has also been reported [9]. More recently, the possibility of combined SFE and derivatization of polar compounds has been explored [10–13]. This latter approach further reduces sample preparation time and at the same time enhances the extractability of polar compounds since derivatives are in general less polar than their parent compounds. Our work on resin and fatty acids [8] demonstrated that this one-step technique can be applied to the rapid screening of the acids in sediment samples. In this report, we shall describe a rapid and quantitative method using an *in situ* extraction/derivatization technique for the determination of PCP and other chlorophenols in soils contaminated by the wood-preserving chemical.

EXPERIMENTAL

All chlorophenol standards were supplied by Supelco. Acetic anhydride and triethylamine were purchased from Aldrich. The anhydride was triple-distilled and the fraction of b.p. 138–140°C was collected and used. Distilled-in-glass solvents were supplied by Burdick & Jackson. Carbon dioxide (SFE grade) with a helium head pressure of 10.5 MPa was obtained from Scott Specialty Gases and Linde.

Stock solutions of individual chlorophenols at 1000 μg/ml were prepared in acetone. Mixtures of the 14 chlorophenols (Table I) at 10 and 50 μg/ml were prepared for the spiking of soil samples and the preparation of calibration standard. A mixture of 2,4-dibromophenol and 2,4,6-tribromophenol at 10 μg/ml, also in acetone, was prepared as a surrogate standard.

TABLE I

PRECISION AND ACCURACY OF THE *IN SITU* SFE/DERIVATIZATION PROCEDURE FOR THE DETERMINATION OF CHLOROPHENOLS IN SPIKED SAMPLES

Mean and standard deviation of six replicate determinations.

Chlorophenol	Recovery (%)	
	0.5 μg/g	5.0 μg/g
2,6-Dichlorophenol	97 ± 7	93 ± 5
2,4-Dichlorophenol	92 ± 7	93 ± 6
3,5-Dichlorophenol	96 ± 8	97 ± 6
2,3-Dichlorophenol	87 ± 6	89 ± 5
3,4-Dichlorophenol	98 ± 6	89 ± 6
2,4,6-Trichlorophenol	93 ± 7	102 ± 5
2,3,6-Trichlorophenol	91 ± 6	97 ± 4
2,3,5-Trichlorophenol	101 ± 4	98 ± 4
2,4,5-Trichlorophenol	101 ± 6	101 ± 5
3,4,5-Trichlorophenol	94 ± 3	90 ± 4
2,3,5,6-Tetrachlorophenol	90 ± 6	95 ± 5
2,3,4,6-Tetrachlorophenol	101 ± 7	103 ± 5
2,3,4,5-Tetrachlorophenol	104 ± 4	93 ± 4
Pentachlorophenol	96 ± 6	102 ± 5

For consistency, all samples were prepared in the following manner prior to extraction. Two layers of Whatman GF/C filters cut to the diameter of the extraction thimble were placed on top of the bottom thimble cap to minimize contamination and plugging of the frit. The 7-ml thimble was then filled with 200 mg of Celite followed by 1 g of sample, which was previously mixed and ground. A 50-μl volume of the above surrogate solution and 30 μl of triethylamine were spiked to the soil sample. If the soil was completely dry, 50 μl of water (equivalent to a moisture content of 5%, w/w) was also added directly to the sample. The thimble was shaken on a vortex mixer for 15 s after addition of each liquid. The sample was topped by another 200 mg of Celite and 30 μl of acetic anhydride were added before it was subject to supercritical carbon dioxide extraction. In the case of recovery experiments, samples were prepared as described above except that the surrogate solute also contained a known amount of the 14 chlorophenols.

All extractions were performed by a Hewlett-Packard 7680A SFE module using supercritical carbon dioxide of a density of 0.8 g/ml (37.2 MPa) and a flow-rate of 2.0 ml/min. Static and dynamic

extractions of 5 min each were carried out and the extraction chamber temperature was maintained at 80°C during this time. An octadecylsilane (ODS) trap, used for the collection of sample extracts, was kept at 15 and 45°C, during the extraction and rinsing stages, respectively. SFE extracts from the trap were eluted by hexane in two 1.2-ml fractions.

The derivatized extract was partitioned with 3 ml of 1% potassium carbonate solution by vortexing in a centrifuge tube for 1 min. This step removed the acetic acid formed in the acetylation reaction and the excess acetic anhydride reagent: both of them could lead to chromatographic problems if the uncleaned extracts were analyzed. The hexane extract was then transferred to a short (3 cm) anhydrous sodium sulfate column and a 5 cm 5% deactivated silica gel column prepared in tandem using disposable Pasteur pipettes for further cleanup. The columns were first eluted with 5 ml of hexane and this fraction was discarded. The acetyl derivatives of chlorophenols were removed from the column by elution with 10 ml of light petroleum (b.p. 30–60°C)–dichloromethane (1:1). This was followed by solvent exchange into 5 ml or other suitable volume of iso-octane.

For comparison of SFE results, steam distillation of soil samples was also performed. A 1-g amount of soil was stirred with 50 ml of a 1% solution of potassium carbonate for 10 min in a 500-ml round-bottom flask. A 1-ml volume of acetic anhydride was added and stirred for another 10 min. The mixture was steam distilled for 1 h into 3 ml of hexane in the condenser according to the method developed by Veith and Kiwus [2]. The acetates were then cleaned up as described anbove except that the silica gel column cleanup was omitted. A commercial standard reference soil sample (SRS 103–100) supplied by Fisher Scientific was used in the comparison study.

Chromatographic analysis was carried out with a Hewlett-Packard 5890 Series II gas chromatograph equipped with an electron-capture detector and a split-splitless injection port. Splitless injection (1 μl) was made by a HP 7673 autosampler onto a 25 m × 0.2 mm I.D. HP-5 fused-silica column. The initial oven temperature was 70°C (0.75 min hold) and it was programmed to 120°C at 30°C/min and then to 200°C at 2°C/min. Splitless time was 0.75 min. Hydrogen was the carrier gas and the column head pressure was 105 kPa. Instrument control and data acquisition were achieved by a personal computer running the HP 3365 ChemStation software in the Microsoft Windows environment.

To calibrate the instrument, a concentrated mixture of the acetyl derivatives of chlorophenols was prepared by an aqueous acetylation of a known amount of chlorophenols according to established procedures [1,14]. Quantitation of chlorophenols in soil samples was performed by an external standard method, using appropriate dilutions of the above mixture with iso-octane.

RESULTS AND DISCUSSION

In a recent report, free PCP was extracted from soil in 60 min using supercritical carbon dioxide at 31.0 MPa and 70°C [9]. In this case, 10% (w/w) of water was added to the sample as a modifier. Quantitative recovery of PCP from soil was obtained in our laboratory by a 15-min extraction with carbon dioxide at 37.2 MPa and 80°C, in the presence of the same amount of modifier. Also, we found that the same approach applied to the extraction of the di-, tri- and tetrachlorophenols as well although the recovery of the less chlorinated phenols were low (60 to 80%) under such conditions. Since chlorophenols are routinely analyzed by electron-capture detection in the form of acetyl derivatives in our laboratories, the above SFE approach would require an off-line derivatization step. The disadvantage of having an extra step in the procedure can be eliminated if the extraction and derivatization steps can be combined into one.

Chlorophenols in water samples can readily be converted into stable acetyl derivative by an *in situ* acetylation using acetic anhydride and a base such as a carbonate or bicarbonate [14]. Acetyl derivatives of chlorophenols with two or more chlorine atoms are sensitively detected by an electron-capture detector and are more amenable to column cleanup than the free phenols. For these reasons as well as the fact that the acetyl derivatives are easily formed and stable under the SFE conditions, they are the most appropriate choice for this work.

Similar to the aqueous reaction, derivatization of chlorophenols under SFE conditions also required a base. Although the *in situ* acetylation of chlorophenols was working with an aqueous solution of potassium carbonate, quantitative derivatization of

TABLE II

RESULTS OF PCP AND OTHER CHLOROPHENOLS (μg/g) IN A REFERENCE SOIL SRS 103-100 by SFE AND STEAM DISTILLATION

N.D. = No data.

Chlorophenol	Steam distillation (this work) ($n = 3$)	SFE (this work) ($n = 6$)	SFE (ref. 9) ($n = 3$)
2,3,5-Tri-	0.40 ± 0.01	0.36 ± 0.01	N.D.
2,3,5,6-Tetra-	14.4 ± 0.4	13.9 ± 0.3	N.D.
2,3,4,6-Tetra-	20.6 ± 0.4	20.2 ± 0.3	N.D.
2,3,4,5-Tetra-	1.9 ± 0.1	1.8 ± 0.1	N.D.
PCP	1499 ± 67	1483 ± 93	1361

all phenols could only be achieved in the presence of triethylamine. Presumably, the inorganic base is less effective than the organic base since the former is less soluble in supercritical carbon dioxide and thus less available for the reaction. In order to have the highest recovery of the acetyl derivatives, approximately equal volumes of acetic anhydride and triethylamine should be used. A large excess (250 μl or more) of the two reagents was found to be detrimental to the recovery of the derivatives. A

chamber temperature of 80°C was chosen since, at this temperature, a 10-min extraction was enough for the complete recovery of the chlorophenols in soil. On the contrary, only 60 and 90% of the PCP could be recovered in 10 min if the chamber temperature was set at 40 and 60°C, respectively.

In order to evaluate the efficiency of the *in situ* SFE/derivatization procedure, the recovery of chlorophenols from clean soil samples fortified at different levels was determined. Basically, recoveries of 90% or above were obtained in the 0.5 and 5 μg/g range for PCP and other chlorophenols (Table I). The results suggested that this method is also applicable to the quantitative determination of di-, tri- and tetrachlorophenols if they are present in the soil samples.

The ruggedness of the SFE method was again tested with a standard reference soil sample (SRS 103-100) naturally contaminated by PCP. In a side-by-side comparison, our results for PCP and other chlorophenols generated by the *in situ* SFE/derivatization procedure for this sample are nearly identical to those obtained by the steam distillation procedure, indicating completeness of extraction and derivatization (Table II). Both techniques also showed similar degree of precision as indicated by
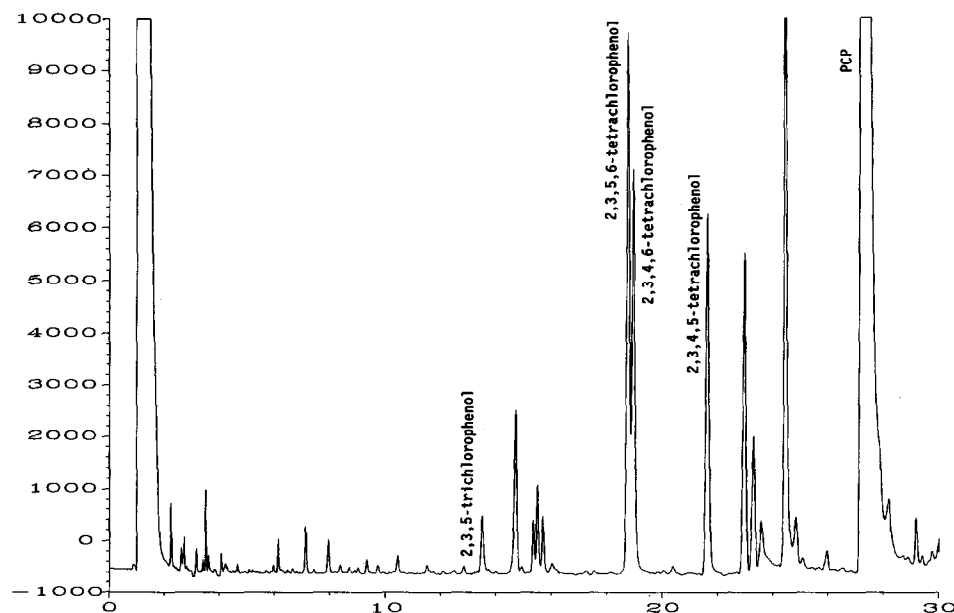

Fig. 1. Gas chromatography–electron-capture detection of the acetyl derivatives of chlorophenols in a contaminated soil sample prepared by *in situ* SFE and derivatization.

the standard deviation in replicate determinations. It should also be noted that our PCP result for this reference sample (1483 μg/g) is more comparable to that obtained by the non-derivatized SFE approach (1361 μg/g) [9] than the rather ambiguous certified value (965 ± 374 μg/g) furnished by the supplier.

This new procedure is being evaluated for the determination of chlorophenols from contaminated soil samples collected in an Ontario site for the preservation of railroad ties and hydro poles. The texture of the samples varied from light color sandy type to dark color loamy soil. Other than PCP, which contributed 90% (w/w) or more of the total chlorophenols in nearly all cases, tetrachlorophenols and a few trichlorophenols were also detected in the samples analyzed. The levels of chlorophenols in these soil samples varied from *ca.* 100 ng/g for some trichlorophenols to over 1000 μg/g for PCP, indicating the method is applicable to a wide range of concentrations. Again, the SFE results were in good agreement with the steam distillation results in the cases where both techniques were used for cross checking. If the surrogates (bromophenols) were less than 75% recovered, the extraction was repeated. Fig. 1 is an ECD chromatogram of a contaminated soil sample after SFE/derivatization and cleanup. The levels of 2,3,5-trichlorophenol, 2,3,5,6-, 2,3,4,6- and 2,3,4,5-tetrachlorophenol and PCP are 0.12, 0.98, 0.71, 0.55 and 57.8 μg/g, respectively, in the sample. The entire analytical sequence (sample preparation, extraction, derivatization, cleanup, solvent replacement, gas chromatographic analysis and report generation) required approximately 90 min.

CONCLUSIONS

The method described here is suitable for the rapid yet quantitative and specific determination of chlorophenols in soil and sediment samples in the ng/g to μg/g range. This procedure is more efficient and has a wider application than the one reported for the SFE of free PCP from soil. The present SFE method is proven to be a reliable alternative to the established steam distillation procedure since they both produce similar results for real-life samples. The simple analytical procedure results in an extremely short sample turn around time and thus it is most valuable under an environmental emergency situation. It also stands out in environmental friendliness since it consumes much less solvents and chemicals than all existing methodologies involving the derivatization step.

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