Comparison of Supercritical Fluid Extraction with in situ Derivatization and Conventional Extraction Methods for the Analysis of Pentachlorophenol in Wood and Leather

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Abstract

A rapid, efficient method for the extraction of pentachlorophenol (PCP) from wood and leather based on supercritical fluid extraction (SFE) with in situ derivatization is described. PCP is extracted with supercritical carbon dioxide and derivatized in situ with acetic anhydride in the presence of triethylamine under static SFE conditions (50°C, 300 atm). Light petroleum traps are used for analyte collection during the dynamic extraction step. The results are in good agreement with those obtained from different laboratories using conventional methods. When using SFE with in situ acetylation, determination of PCP in wood and leather can be carried out in less than 3 h.

Introduction

Pentachlorophenol (PCP) has been widely used as a biocide in wood protection (1). Due to its slow decomposition rate and toxicity to mammals and fish, PCP is an environmental concern. In 1989, the German PCP prohibition order established an upper limit of 5 mg/kg for the PCP content in wood, leather, and other matrices (2,3), but despite these regulations, PCP is still detectable in leather goods (e.g., shoes) and wooden articles.

Various methods are used to determine PCP in wood. Therefore, the results might be incorrect or incomparable; this assumption was confirmed by an interlaboratory evaluation study in which the deviations from the expected value ranged from –57% to 600% (4). Due to these varying results, the aim of this work was the development of a method for the precise determination of PCP in wood samples.

PCP can interact strongly with the active sites of the matrix. As a consequence, many organic solvents fail to extract PCP quantitatively (4). Due to their unique properties (low viscosity, almost no surface tension), supercritical fluids (SFs) might efficiently penetrate the wood matrix, and because the diffusion coefficients of solutes are usually higher in SFs than in liquids, SF extraction (SFE) could be an efficient alternative to conventional methods. SFE with in situ derivatization (SFE-D) has been used successfully to determine PCP in leather samples (5,6). With this approach, extraction and derivatization of PCP are performed in one step. Apart from this, the derivatization reagents enhance the efficiency of SFE because the acetyl derivative of PCP is less polar than the free compound and therefore more amenable to extractions with the nonpolar carbon dioxide. In addition, the derivatization reagents might act as modifiers which interact with the active sites of the matrix and, in this way, enhance the extraction efficiency.

To our knowledge, such an approach has not yet been applied to PCP determination in wood; therefore, the applicability of this method was investigated in this study. For both wood and leather, the SFE results were compared to those obtained by other laboratories using conventional methods.

Experimental

Samples and standards

All solvents as well as acetic anhydride and triethylamine were purchased from Merck (Darmstadt, Germany) in the highest purity available. The anhydride was triple-distilled and the fraction that had a boiling point between 138 and 140°C was used. Carbon dioxide with a helium head pressure of 100 atm (1 atm = 101 325 Pa) was supplied by Westfalen Gas (Münster, Germany). Stock solutions of PCP (Alltech, Unterhaching, Germany), 2,4,6-tribromophenol (TBP), and hexachlorobenzene (HCB) (Aldrich, Steinheim, Germany) were prepared in toluene. For calibration of the gas chromatography–electron-capture detector (GC–ECD) instrument, PCP and TBP were derivatized according to an established procedure (7). Appropriate dilutions of acetylated PCP (0.03–0.36 µg/mL) were then prepared in toluene, each containing 0.2 µg of acetylated TBP per milliliter and 0.3 µg of HCB per milliliter. During method development, acetylated 3-methyl-4-nitrophenol was used as an additional internal standard to be added together with HCB (6).

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As was expected, both of these yielded the same results; therefore, only the HCB results were mentioned in the text.

SFE

An SFE system (5) that had been built in our laboratory was used for all extractions. The extraction cells (3.5 mL, 5 cm × 9.4-mm i.d.) (Dionex, Idstein, Germany) were filled with silanized glass-fiber wadding (Macherey-Nagel, Düren, Germany) and 0.5–1 g of the wood sample (or 0.7–1.8 g of the leather sample), which was then spiked with the internal standard TBP in acetone in such an amount that the final concentration was the same as it had been in the multilevel internal standard calibration procedure (0.2 µg/mL).

After the addition of 100 µL of triethylamine (TEA), the loaded cell was heated to 50°C for 5 min prior to the addition of 400 µL acetic anhydride through a valve. Extractions were then carried out at 250 or 300 atm for 10 min in the static mode and for about 25 min in the dynamic extraction mode (each extraction was performed with 30 mL of liquid carbon dioxide).

Ice-cooled dual-chamber trapping vials (8) filled with 15 mL of light petroleum (boiling point, 40–60°C) were used for analyte collection. The extracts were shaken out with 3 mL of 2% potassium hydrogen carbonate solution for 1 min. Under a gentle stream of nitrogen, the extracts were concentrated to about 2 mL. The extracts were cleaned on 0.55 g of silicagel, and the acetylated phenols were eluted with 9 mL of toluene. Finally, they were filled up to 10 mL in a volumetric flask. A more detailed description of the analytical sequence is given in references 5 and 6.

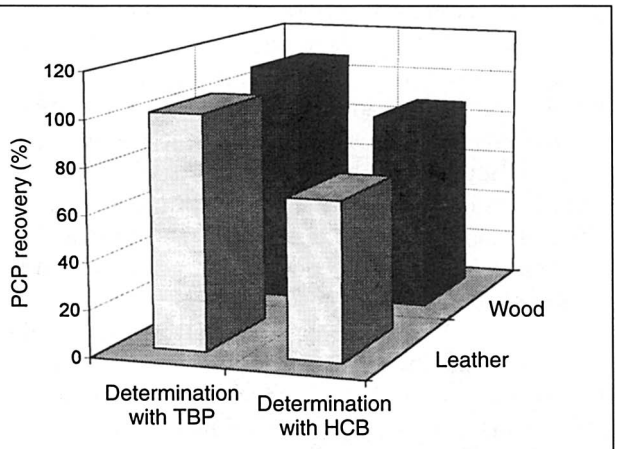

Figure 1. Recovery of PCP from spiked leather and wood. Leather, eight replicates; wood, three replicates. RSD = 6–9% (leather), 2% (wood).

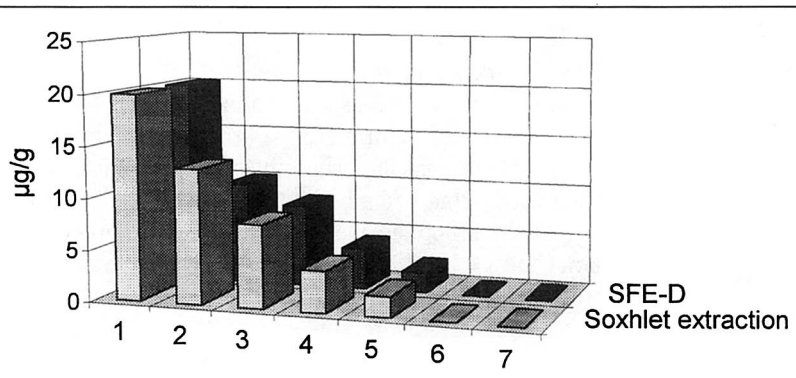

Figure 2. Results of the interlaboratory comparison study of PCP in leather samples. Soxhlet extraction with methanol–acetone (results of the "Bremer Umweltinstitut", Bremen, Germany): two replicates; internal standard, TBP; RSD of the method, 9.6% (determined by 10-fold extraction of one sample). SFE-D was carried out as described in the text (internal standard, TBP; 4–9 replicates, depending on the available amount of the leather samples). SDs: sample 1, 1.1 µg/g; sample 2, 3.7 µg/g (wet-blue leather; this sample had to be dried in air for days before SFE); samples 3–5, 0.3–0.9 µg/g; samples 6–7, PCP content was less than 0.05 µg/g.

GC analysis

Chromatographic analysis was carried out using a Varian series 3300 GC equipped with a split–splitless injection port, an ECD (Varian, Darmstadt, Germany), and either a J&W DB-1701 (30 m × 0.32-mm i.d., 0.25-µm film thickness) (J&W Scientific, Folsom, CA) or a Permabond SE-54-DF-0.25 (25 m × 0.32-mm i.d.) (Macherey-Nagel) capillary column. Both of them were equipped with a 2-m deactivated fused-silica precolumn (Macherey-Nagel). The following temperature programs were applied. The DB-1701 column was initially heated to 80°C. Temperature was then increased to 165°C at 20°C/min, to 182°C at 2.5°C/min, to 200°C at 10°C/min (held for 1 min), and finally to 240°C at 20°C/min. The SE-54 column was initially heated to 80°C. The temperature was then increased to 130°C at 20°C/min, to 150°C at 5°C/min, to 200°C (held for 1 min) at 10°C/min, and finally to 240°C at 20°C/min (held for 1 min). Nitrogen was used as the carrier gas with a column head pressure of 1.05 atm. Split injections (1:20) were performed with a Dynatech GC-411V autosampler (Analyte GmbH, Müllheim, Germany). Dionex AI-450 software was used for data acquisition and analysis.

Results and Discussion

Matrix effects are known to be an important problem in SFE (9–11). Hence, the extraction efficiency might vary enormously if a single method is applied to different matrices; this is also valid for SFE with in situ derivatization. The extraction of PCP from soils was shown to be complete within 10 min using small amounts of TEA and acetic anhydride (30 µL of each) (12), whereas in the case of leather (5,6), an extraction time of about 30 min and a large excess of derivatization reagents (100 µL TEA and 400 µL acetic anhydride) were required for quantitative extractions. In addition, sample cleanup might have to be adjusted due to problems with coextractives (6).

In the case of uncontaminated leather samples that had been spiked with 23.6 µg of PCP and 30.2 µg of TBP in acetone, SFE derivatization yielded quantitative PCP recovery (Figure 1), especially when TBP was used as an internal standard. Because

TBP was added to the extracts prior to SFE, it was able to compensate analyte losses (assuming that PCP and TBP behave analogously). The second internal standard, HCB, was added just before GC analysis and therefore only indicated the amount of PCP that was actually in the extract. Hence, a discrepancy between the results obtained with HCB and TBP indicated analyte losses during sample preparation.

The results of PCP extractions from an inert matrix (C_{18} material) (5) proved the applicability of the internal standard TBP because the PCP recovery in this case was 98%. Even with HCB, almost quantitative PCP recovery (88%) was obtained for spiked C_{18}; therefore, systematic analyte losses seem to play a subordinate role. The enhanced discrepancy between the TBP and HCB results that had been observed for spiked leather samples must therefore be a consequence of matrix effects. To avoid low results, the use of TBP is therefore highly recommended in the routine analysis of PCP in leather samples.

In the next step, the applicability of SFE with in situ acetylation was examined using spiked wood samples. Therefore, an uncontaminated wood sample was spiked with 23.6 µg of PCP and 30.2 µg of TBP in acetone. Again, HCB was used as an additional internal standard to be added just before GC analysis.

Because PCP was quantitatively recovered from spiked wood (Figure 1), the method also seemed to be applicable for this purpose. The discrepancy between the TBP and HCB results was lower than for spiked leather samples, which indicated that matrix effects played a subordinate role in this case.

Spiked samples cannot replace naturally contaminated ones because the interactions between the analytes and the active sites of the matrix are usually weaker with spiked samples. First, the suitability of the SFE method was examined using "naturally" contaminated leather samples. Therefore, different types of leather were first analyzed by a routine laboratory using a Soxhlet extraction method that had yielded good results in an interlaboratory ring test. After that, SFE with in situ derivatization was used to determine PCP in these leather samples. By doing this, at least the comparability of the results obtained by SFE derivatization with those of a conventional method was evaluable. The results (Figure 2) indicated an excellent agreement between the two procedures. Hence, SFE derivatization seems to be a powerful alternative to conventional methods, especially because it is very time-efficient and ecologically harmless.

To investigate the efficiency of SFE derivatization for the determination of PCP in "naturally" contaminated wood samples, several wood samples were analyzed for which the PCP content had already been determined by other laboratories using conventional methods (both methods, e.g., alkaline extraction under reflux [13], had yielded good results in interlaboratory ring tests before). The results are presented in Table I.

The SFE results were in good agreement with those of the routine laboratories for PCP levels in the range required to control the observance of the PCP prohibition order. As the PCP content increases, SFE with in situ acetylation might yield higher recoveries, but it should be noted that, with the conventional method B that was used in this case, no internal standard was employed.

Apart from this, homogeneity of the sample is a very important aspect in the analysis of wood samples because the amount of PCP decreases dramatically in inner layers (4). If possible, the layer to be analyzed should be ground into particles less than 1 mm in diameter, but for this part of the study, only wood shavings of different sizes were available.

In the next step, the SFE efficiency was evaluated in an interlaboratory ring test. In this case, the samples were ground into particles less than 1 mm in diameter and were well-homogenized. All 10 participants used their in-house method and/or a standard method based on alkaline extraction with 1M potassium hydroxide under reflux (13). Figure 3 shows a chromatogram of an SFE derivatization extract from the first wood sample. A comparison of the SFE results from our laboratory and the results of the ring test is presented in Table II.

The SFE results were in good agreement with the results of

Table I. Results of the Interlaboratory Evaluation Study

	PCP content (µg/g)		
		SFE with in situ derivatization*	
Sample	Conventional method	Determination with TBP	Determination with HCB
1	A 3.9	3.0	1.9
2	A 4.4	4.4	3.4
3	B 2.2	2.8	1.0
4	B 0.4	0.4	0.4
5	B 31.6	39.2	31.4
6	B 38.8	76.1	65.6

With method A (results of the "Institut Fresenius", Dortmund, Germany), TBP was used as an internal standard and added prior to the extraction of PCP; RSD of method A was 7% (determined for samples spiked with PCP at 0.03 µg/g, five replicates).
With method B (13) (results of the "Analytik-Service Gesellschaft mbH" [ASG], Neusäss, Germany), no internal standard was used; RSD of method B was 2–15% (depending on the PCP content; these results were determined in an interlaboratory ring test, three replicates).

* 2–5 replicates (depending on the available amount of the wood sample). SD for determination of PCP with TBP (or HCB): sample 1, 0.9 (1.1) µg/g; sample 2, 1 (1.2) µg/g; sample 3, 0.3 (0.3) µg/g; sample 4, 0.01 (0.08) µg/g; sample 5, 10 (7) µg/g; sample 6, 3.9 (3.8) µg/g.

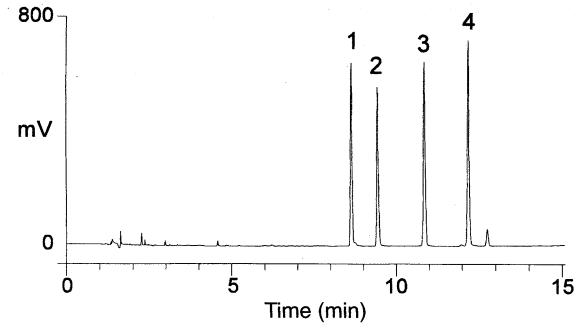

Figure 3. GC–ECD chromatogram of a wood extract obtained by SFE-D. Chromatographic conditions are described in the text (DB-1701 column). Peaks: 1, 3-methyl-4-nitrophenol (acetyl derivative); 2, HCB; 3, TBP (acetyl derivative); 4, PCP (acetyl derivative).

Table II. Comparison of SFE Results and Mean Values of the Interlaboratory Ring Test

	SFE with in situ derivatization		
	PCP determination with TBP	PCP determination with HCB	Interlaboratory ring test
Sample	Mean ± SD (µg/g)	Mean ± SD (µg/g)	Mean ± SD (µg/g)[§]
1	57 ± 5*	49 ± 2*	53 ± 15
2	0.13 ± 0.07[†]	0.13 ± 0.03[†]	0.2 ± 0.1
3	273 ± 22[‡]	212 ± 18[‡]	235 ± 68

* Five replicates.
[†] Three replicates.
[‡] Six replicates.
[§] Outliers were not taken into consideration; 16–19 replicates.

the interlaboratory ring test in which numerous methods were involved. Again, PCP determination with the internal standard TBP yielded somewhat higher recoveries, but they did not exceed the confidence ranges of the mean values. Because details about the in-house methods used in the interlaboratory ring test were not available, it is difficult to interpret these results. However, it should be emphasized that SFE with in situ acetylation is a very efficient alternative to conventional methods.

In this study, it was shown that a single SFE derivatization method can be successfully applied to leather and wood samples despite the enormous differences in matrix composition. For both matrices, the SFE results were in excellent agreement with those of routine laboratories using conventional methods. When using SFE with in situ acetylation, the determination of PCP was performable within 3 h, and less than 50 mL of organic solvents was required.

Acknowledgments

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