Table 5
Mean values and standard deviations of the area-percents of selected methyl esters of carboxylic acids in Unicid® 550

Carbon number	Mean ± S.D. ($n = 6$)
16	0.234 ± 0.0689
20	0.519 ± 0.0403
24	1.20 ± 0.0692
28	2.31 ± 0.0822
32	3.61 ± 0.0691
36	4.93 ± 0.00902
40	5.83 ± 0.109
44	5.98 ± 0.104
48	5.41 ± 0.108
52	4.36 ± 0.0985
56	3.14 ± 0.0906

lar mass distribution, with reactivity and physical properties of Unicid™ acids.

Acknowledgements

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Determination of pentachlorophenol in leather using supercritical fluid extraction with in situ derivatization

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Abstract

An in situ supercritical fluid extraction (SFE) and derivatization procedure for the determination of pentachlorophenol (PCP) in leather is described. PCP was extracted from leather with supercritical carbon dioxide and in situ derivatized with acetic anhydride in the presence of a base (e.g., triethylamine). The influence of several extraction and derivatization parameters (e.g., pressure, temperature, extraction time in the static and dynamic extraction mode, amount of the base and of acetic anhydride) on the extraction efficiency has been investigated. Since the leather sample had no certified PCP content, the SFE results were compared with those obtained by Soxhlet extraction with methanol. With SFE instead of conventional Soxhlet extraction, the overall time required for sample preparation, extraction, derivatization, evaporation, clean-up and analysis steps can be reduced from about 2 days to approximately 3 h.

1. Introduction

Since its commercial introduction in 1936, pentachlorophenol (PCP) has found world-wide application, e.g., in commercial wood treatment (as a preservative, insecticide and microbiocide), for paper production (for reduction of slime), in leather industry (as a preservative and fungicide) and in agriculture (as an herbicide and insecticide) [1–3]. Therefore, human exposure to this chemical cannot be prevented. PCP is an environmental concern as it is toxic to fish and mammals [1]. However, because of its broad efficiency spectrum and the low cost of production, PCP is still in use.

In 1989, the German pentachlorophenol prohibition order has established an upper limit of 5 mg/kg for the PCP content in leather and other matrices [4,5].

Solvent extraction (e.g., Soxhlet extraction) or steam distillation techniques are widely used for extractions of PCP from leather [6], although these methods are time-consuming. In the case of solvent extractions, large amounts of organic solvents are required and several clean-up steps are necessary to remove coextractives. Both techniques require an additional derivatization step if PCP is to be analyzed by gas chromatography (GC) in the form of, e.g., an acetyl derivative. However, the risk of sample losses increases with each step in the process.

Therefore, the aim of this work was to develop

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a simple, rapid and precise method for the determination of PCP in leather samples.

Supercritical fluid extraction (SFE) has been proved to be an efficient alternative to conventional methods for extractions of polychlorinated biphenyls, polynuclear aromatic hydrocarbons, dioxins and chlorobenzenes from soils, sediments and other solid matrices [7–13]. However, quantitative extractions of polar analytes (e.g., phenols) require the addition of a polar modifier (e.g., methanol) to the non-polar carbon dioxide. Recently, extractions of phenols from soils and sediments have been performed by in situ extraction and chemical derivatization under SFE conditions [14].

No extra step is required for derivatization of the analytes. In addition, the polarity of the analytes is usually decreased by derivatization, and therefore they are easier to extract, and they become more amenable to subsequent column clean-up than the free compounds.

In this study, a method for the determination of pentachlorophenol in leather being based upon SFE with in situ acetylation is presented. The influence of individual extraction and derivatization parameters on the extraction efficiency was investigated. The results of the SFE–derivatization procedure were compared with those obtained by conventional Soxhlet extraction.

2. Experimental

2.1. Samples and standards

Pentachlorophenol was obtained from Alltech (Unterhaching, Germany), 2,4,6-tribromophenol (TBP) from Aldrich (Steinheim, Germany) and 2-methyl-4-nitrophenol (2-M-4-NP) from Dr. Ehrenstorfer (Augsburg, Germany). Isopropylamine was purchased from Fluka (Neu-Ulm, Germany). All solvents as well as acetic anhydride, triethylamine (TEA) and potassium hydrogencarbonate were obtained from Merck (Darmstadt, Germany) in the highest purity available. The anhydride was triple-distilled and the fraction of b.p. 138–140°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 (8 mg/ml) and the internal standard 2,4,6-tribromophenol (18 mg/ml) have been prepared in toluene. For spiking purposes, acetone solutions containing 0.5 mg PCP/ml and 0.7 mg TBP/ml were prepared. Acetylated 2-methyl-4-nitrophenol ("2-M-4-NPAc") was used as an internal standard for GC analysis and it was prepared by aqueous derivatization according to an established procedure [15]. For calibration of the instrument, PCP and TBP also had to be derivatized. Stock solutions of acetylated PCP (9 μg/ml), TBP (70 μg/ml) and 2-M-4-NP (220 μg/ml) were prepared in toluene.

2.2. Extraction of PCP from leather

Soxhlet extractions

A 70-ml Soxhlet extractor with 100 × 25 mm I.D. extraction thimbles (Schleicher & Schüll, Dassel, Germany) and a 100-ml round-bottomed flask was used for all extractions. The extraction process was based upon a procedure described in [16]. Each extraction was carried out with 6 g of the leather sample and 80 ml of methanol for 8 h in the dark. The internal standard 2,4,6-tribromophenol was added to the extract in such an amount that the final concentration was the same as it had been in the calibration procedure of the GC–ECD system (0.2 μg/ml). After concentration and several liquid–liquid partitioning-steps, the extract was cleaned-up on silica gel. Finally, derivatization of PCP was performed in aqueous solution as described by Lee et al. [15]. To determine the amount of PCP being actually in the extract, a defined volume of the internal standard 2-M-4-NPAc was added to appropriate dilutions of the extracts in toluene for GC–ECD analysis.

Supercritical fluid extractions

All supercritical fluid extractions were performed with an in-house-built SFE system which consists of a syringe pump, a metal block (especially designed to the form of the extraction

cells) which was heated with water, a thermostat which controls the temperature of the water bath, several valves that allow extractions in the static and dynamic mode and a heated restrictor (PEEK-capillary; 10 cm × 125 μm I.D.).

For recovery experiments, the extraction cells (Dionex, Idstein, Germany; 3.5 ml; 5 cm × 9.4 mm I.D.) were filled with silanized glass-fibre wadding (Macherey-Nagel, Düren, Germany) and about 1.6 g of Chromabond C_{18} endcapped (Macherey-Nagel). The material was spiked with 50 μl of the PCP solution containing 473 μg/ml acetone and 40 μl of the internal standard TBP (710 μg/ml acetone). The solvent was allowed to evaporate. After addition of 100 μl of triethylamine, the loaded cell was heated in the metal block to 50°C for 5 min before 400 μl of acetic anhydride was added via an extra valve. At a pressure of 300 atm, the sample was then extracted for 10 min in the static and for about 15 min (corresponding to 20 ml of carbon dioxide, measured in its liquid state) in the dynamic extraction mode.

Analyte collection after off-line SFE was performed with chilled dual-chamber trapping vials [17] which were filled with light petroleum.

For extractions of PCP from leather, the extraction cells were filled with 1.8 g of the leather sample which was cut up (in the cm^2 range), and after addition of the internal standard TBP, extractions were carried out as described above (otherwise the parameters are mentioned in the text).

2.3. Clean-up

The extracts were partitioned with 3 ml of 2% potassium carbonate solution in a separation funnel for 1 min. This step was necessary for the removal of the excess acetic anhydride and the acetic acid formed in the derivatization step. Both of these can lead to chromatographic problems if the uncleaned extracts are analyzed [14].

Under a gentle stream of nitrogen, the solvent was evaporated to about 2 ml. A clean-up column [Pasteur pipette (23 × 0.5 cm I.D.) filled with silanized glass-fibre wadding, 0.55 g of silica gel (30–60 μm; J.T. Baker, Gross-Gerau, Germany) and 0.25 g of anhydrous sodium sulphate (Merck)] was rinsed with 5 ml of *n*-hexane. The light-petroleum extract was transferred to the clean-up column which was finally rinsed with 0.5 ml of *n*-hexane. This fraction was discarded. Elution of the acetylated chlorophenols was performed with 9 ml of toluene. In a volumetric flask the extract was filled up to 10 ml with toluene. For GC–ECD analysis a defined volume of 2-M-4-NPAc was added to appropriate dilutions of the extracts.

2.4. Gas chromatographic analysis

Gas chromatographic analysis was carried out with a Varian (Darmstadt, Germany) Series 3300 gas chromatograph equipped with a split/splitless injection port and an electron capture detector. Split (1:20) injection (1 μl) was performed with a Dynatech GC-411V autosampler. The 25 m × 0.32 mm I.D. Permabond SE-54-DF-0.25 capillary column (Macherey-Nagel, Düren, Germany) and the 2-m deactivated fused-silica precolumn (Macherey-Nagel) were initially heated to 80°C. 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 230°C (held for 5 min) at 30°C/min. Nitrogen was used as carrier gas with a column head pressure of 1.05 atm. Dionex AI-450 software was used for data acquisition and analysis. For instrument calibration, acetyl derivatives of PCP, TBP and 2-M-4-NP were prepared as described above. Quantification was performed according to the multilevel internal standard calibration method. Therefore, appropriate dilutions of acetylated PCP (0.03–0.36 μg/ml) were prepared in toluene, each containing 0.2 μg of acetylated TBP/ml and 0.8 μg of 2-M-4-NPAc/ml.

3. Results and discussion

SFE with in situ derivatization is an efficient alternative to conventional SFE techniques as this approach further reduces sample preparation

Table 1
Recovery of PCP from spiked C_{18}

	PCP added (μg)	PCP recovered $\bar{x} \pm \sigma$ (μg)	Recovery $\bar{x} \pm \sigma$ (%)
PCP, determined with TBP	23.6	24.3 ± 1.2	103 ± 5
PCP, determined with 2-M-4-NPAc	23.6	24.8 ± 1.3	105 ± 7

Abbreviations as in Fig. 1. Extractions were carried out as described in the text. $n = 10$.

time and at the same time enhances the extractability of polar analytes by reducing their polarity [14].

In order to evaluate the efficiency of the in situ SFE–derivatization method (especially of analyte collection and clean-up steps), the recovery of PCP from an inert matrix (C_{18}) being spiked at a known level has been determined, and the results are presented in Table 1.

As PCP was completely recovered from spiked C_{18}, no sample losses had occurred during sample preparation.

In naturally contaminated matrices, interactions between the analytes and the active sites of the matrix can be very strong. In order to investigate the influence of matrix effects on the PCP-recovery from leather, the described SFE procedure was applied to a "naturally" contaminated leather sample. Fig. 1 shows a chromatogram of a leather extract after SFE–derivatization and clean-up.

Since the leather sample used for this study had no certified PCP-content, conventional Soxhlet extraction with methanol (see above) was used to determine it. The presence of PCP has been proved by GC–MS (Fig. 2). 2,3,4,6-Tetrachlorophenol has also been detected in the leather sample. A comparison between the results obtained by Soxhlet extraction and SFE is presented in Fig. 3.

The two methods yielded comparable results. Despite the differences in extraction, derivatization and clean-up steps, determination of PCP with the internal standard TBP yielded higher recoveries than determination with 2-M-4-NPAc for both methods.

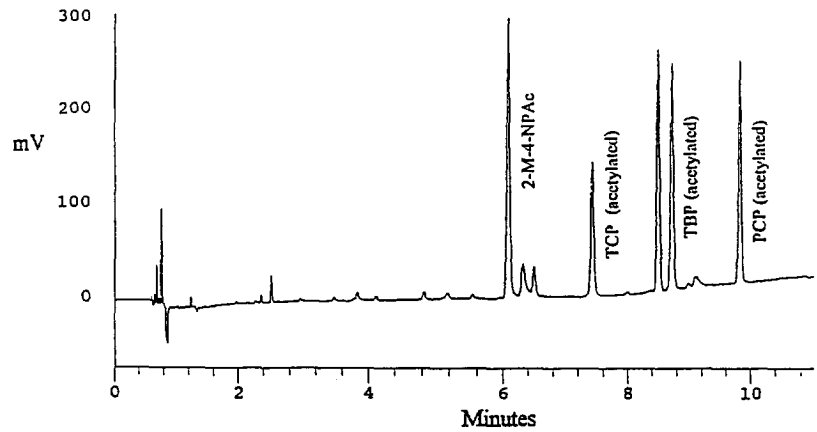

Fig. 1. GC–ECD chromatogram of a leather extract obtained by SFE with in situ acetylation. Chromatographic conditions are described in the text. 2-M-4-NPAc = acetylated 2-methyl-4-nitrophenol; TCP = 2,3,4,6-tetrachlorophenol; TBP = 2,4,6-tribromophenol; PCP = pentachlorophenol.

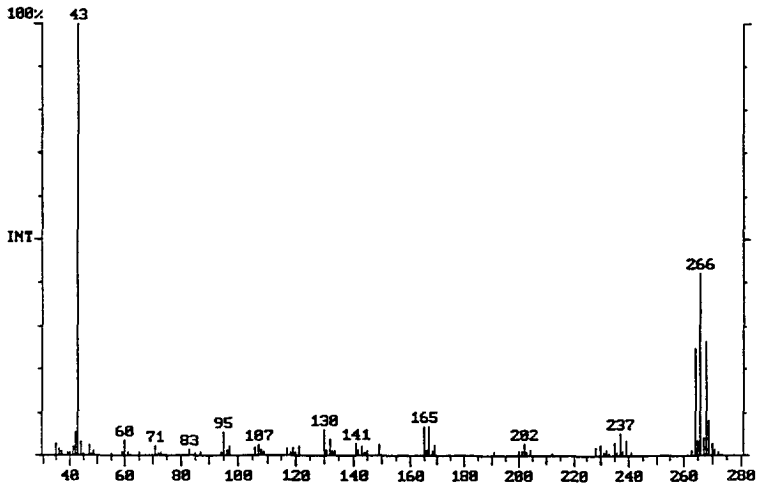

Fig. 2. Mass spectrum of acetylated PCP in a leather extract.

TBP has to be derivatized and extracted like PCP. Therefore, determination with TBP should compensate analyte losses due to incomplete derivatization and/or extraction while determination of PCP with 2-M-4-NPAc (which is added to the extract just before GC analysis) only indicates the amount of PCP being actually in the extract. Differences between these two methods must therefore be a consequence of analyte losses. It should be noted, that according to statistical tests, the results are really different (at least for SFE) although the error bars overlap.

Analyte collection is one of the most problematic steps in SFE, but in this case, quantitative recoveries have been observed for SFE of PCP from spiked C_{18} (note Table 1) using light-petroleum traps. Besides, these traps yielded the best results for PCP extractions from leather when being compared with other trapping methods [18].

Clean-up of the extracts, which is another problematical step in SFE, has been optimized in a previous study [18]. Hence, in the case of SFE, the discrepancy between the two determination methods must have been caused by incomplete extraction and/or derivatization of the analytes. This could be a consequence of matrix effects, since determination with both internal standards yielded comparable results for extractions of PCP from spiked C_{18} (note Table 1).

To optimize the SFE method, influences of individual extraction and derivatization parameters on the recovery of PCP have been investigated in this study.

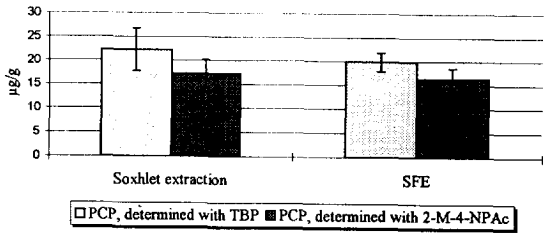

Fig. 3. Results obtained from Soxhlet extraction and SFE. Abbreviations as in Fig. 1. Soxhlet extractions were performed with methanol as described in the text; $n = 8$. SFEs were carried out as described in the text using 20 ml of liquid CO_2 for dynamic extractions; $n = 12$; note Table 3.

3.1. Influence of type and amount of the base and of the amount of acetic anhydride

In water samples, chlorophenols can easily be converted into stable acetyl derivatives using acetic anhydride and a base such as potassium hydrogencarbonate. Therefore, derivatization under SFE conditions was also carried out in the presence of potassium hydrogencarbonate, although quantitative recovery had already been achieved with triethylamine (note Table 1).

According to the water resistance of leather samples, the addition of potassium hydrogencarbonate was problematical. Isopropylamine has also been employed for SFE with in situ acetylation in this study. The results are presented in Fig. 4.

In the case of isopropylamine, determination with the internal standard TBP yielded very high but less reproducible results. This may be a consequence of incomplete recovery of TBP, and this assumption was confirmed by the chromatograms, in which the TBP peak was much smaller than expected. With potassium hydrogencarbonate the results were slightly better. In contrast to this, PCP recoveries being determined with 2-M-4-NPAc almost reached the expected value for these bases. The addition of triethylamine yielded the best results.

Reproducible results were obtained with 100 μl of TEA and 400 μl of acetic anhydride. In the case of PCP extractions from soils with in situ acetylation, the best results were achieved with small amounts of TEA and acetic anhydride (30 μl of each), while the application of a large excess of these chemicals (250 μl and more) deteriorated the results [14]. Hence the influence of the amounts of TEA and acetic anhydride used for derivatization of PCP in leather was investigated in this study.

The amount of the derivatization agents actually influences the efficiency of the method (Fig. 5). In contrast to extractions from soils (see above), addition of 100 μl of TEA and a large excess of acetic anhydride (400 μl) yielded the best results. This might be a consequence of matrix effects since TEA and acetic anhydride may act as modifiers which can change the polarity of the supercritical CO_2 and increase the extraction efficiency by reducing the affinity of the analytes for sorptive sites of the matrix. Apart from this, fatty acids being present in leather samples will also be derivatized, whereby the acetic acid consumption is increased.

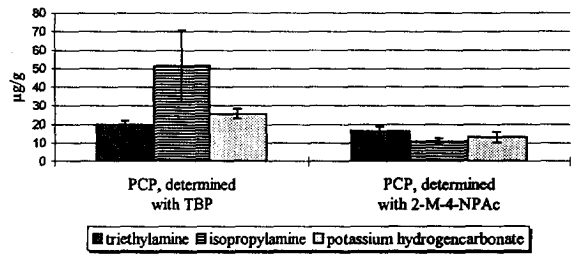

Fig. 4. In situ SFE–derivatization in the presence of different bases. SFE parameters: $p = 300$ atm, $T = 50$°C, $V_{base} = 100$ μl, $V_{acetic\ anhydride} = 400$ μl; extractions were carried out as described in the text; $n = 12$ for triethylamine, $n = 8$ for isopropylamine, $n = 6$ for potassium hydrogencarbonate. Abbreviations as in Fig. 1

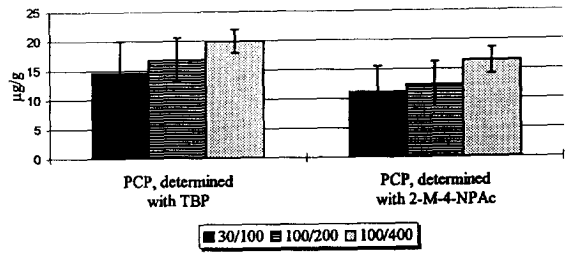

Fig. 5. Results of SFEs performed in the presence of different amounts of TEA and acetic anhydride. In the cases 30/100 and 100/200 amounts of 30 μl (100 μl) of TEA and 100 μl (200 μl) of acetic anhydride were added to the leather sample prior to thermostatization of the cells. Here $n = 6$ and 11, respectively. In the case 100/400 an amount of 100 μl of TEA was added prior to thermostatization of the cell while 400 μl of acetic anhydride was added afterwards (but prior to static extraction) via an extra valve. Here $n = 12$. Abbreviations as in Fig. 1. SFEs were carried out as described in the text.

3.2. Influence of temperature and pressure on the extraction efficiency

The solvent strength of CO_2 strongly depends on the extraction parameters chosen. At a constant temperature, the density of supercritical fluids (which is related to the solvent strength) increases with pressure. In contrast to this, an elevation of temperature at a given pressure results in a decrease in fluid density, but thermal desorption effects, solute diffusivities and vapour pressures are enhanced at the same time.

The presence of acetic anhydride and TEA made it impossible to predict the optimum parameters for PCP extractions from leather. To determine the optimum pressure, extractions were performed at 200 and 300 atm while the temperature remained at 50°C. The syringe pump was not able to produce pressures above 300 atm, so that possible influences of higher

Table 2
Results of SFEs performed at different temperatures and pressures

	50°C, 200 atm[a] $\bar{x} \pm \sigma$ (μg)	50°C, 300 atm[b] $\bar{x} \pm \sigma$ (μg)	70°C, 300 atm[c] $\bar{x} \pm \sigma$ (μg)	90°C, 300 atm[d] $\bar{x} \pm \sigma$ (μg)
PCP, determined with TBP	20 ± 2	20 ± 2	23 ± 4	21 ± 3
PCP, determined with 2-M-4-NPAC	15 ± 2	16 ± 2	17 ± 3	14 ± 2

Abbreviations as in Fig. 1. Extractions were carried out as described in the text.
[a] $n = 10$. [b] $n = 12$. [c] $n = 6$. [d] $n = 6$.

pressures could not be evaluated in this study. The results are presented in Table 2 and they indicate that pressure had almost no effect on the extraction efficiency in this case.

The optimum temperature was determined by extracting the leather at 50, 70 and 90°C while the other extraction and derivatization parameters remained constant. Temperatures higher than 90°C could not be realized since heating of the extraction cells was carried out with hot water.

Temperature influences the extraction efficiency only to a small extent (Table 2). While at 70°C the results seemed to be slightly better than for the other temperatures, the best reproducibilities were obtained for extractions at 50°C. However, only extractions with good TBP recoveries were taken into consideration. Outliers mainly occurred at 70°C, so that reproducibility was actually even poorer for this temperature than indicated in Table 2. Hence the optimum temperature for PCP extractions from leather (at least for the leather sample used for this study) was 50°C.

However, the choice of the optimum temperature and pressure seemed to play a subordinate role for PCP extractions from leather using SFE with in situ acetylation. Thus, once derivatization has occurred, CO_2 seems to be responsible mainly for the elution of acetylated PCP from the leather sample.

3.3. Influence of the extraction time on the extraction efficiency

Quantitative recovery of PCP from C_{18} was observed for extractions being performed for 10 min in the static and for about 15 min (corresponding to 20 ml of liquid carbon dioxide) in the dynamic mode. In the case of soils, static and dynamic extractions for 5 min each were enough to recover PCP quantitatively [14].

Therefore, PCP extractions from leather were performed with various extraction times in the static mode (2, 5, 10 min) and different volumes of CO_2 in the dynamic mode (20 and 40 ml, measured in the liquid state of CO_2, corresponding to an extraction time of about 15 and 30 min).

Extractions with a static extraction step of 5 min seemed to yield the highest recovery of PCP, but only if determination was performed with TBP (Fig. 6). However, higher amounts of PCP being determined with this internal standard may be a consequence of incomplete recovery of the acetylated TBP. Determination with 2-M-4-NPAc yielded the lowest recoveries for SFEs with static extractions of 2 and 5 min. Hence static extraction times of 10 min were necessary for quantitative and reproducible recovery of PCP from leather samples.

The best results were obtained if static ex-

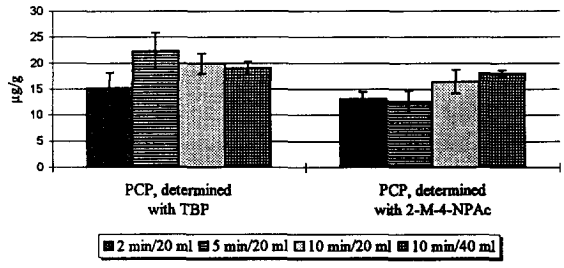

Fig. 6. Results of SFEs performed with various static extraction times and different amounts of liquid CO_2 used for dynamic extractions. Here $n = 5$ (2 min), 7 (5 min), 12 (10 min/20 ml), 3 (10 min/40 ml). Abbreviations as in Fig. 1. Extractions were carried out as described in the text.