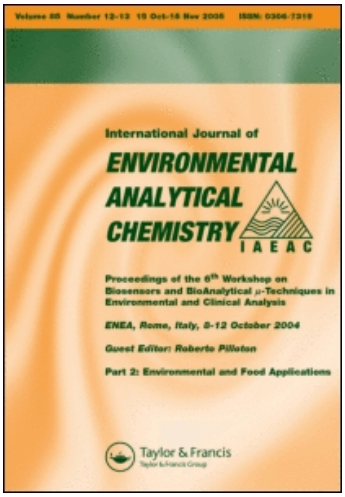

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Determination of Pentachlorophenol in Wood Samples Using Liquid Chromatography With UV Absorbance, Amperometric and Electron-Capture Detection

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The potential of reversed-phase liquid chromatography (RPLC) with UV and amperometric detection (AD), and of normal-phase liquid chromatography (NPLC) with UV and electron-capture detection (ECD) for the determination of pentachlorophenol (PCP) in wood samples has been studied.

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When PCP concentrations of at least 1–5 ppm have to be determined, RPLC-UV and RPLC-AD on C18-modified silica are useful techniques, provided a two- or three-step sample-preparation step is used. NPLC-UV on bare silica columns does not offer any advantage over RPLC-UV. NPLC-ECD on bare silica and with an acidified toluene–hexane mixture as eluent offers good selectivity and sensitivity, as well as satisfactory linearity and reproducibility for the determination of PCP in wood samples down to low ppb levels. Use of the two-step clean-up procedure is sufficient, and even a single-step procedure has been utilized. In the latter case, analysis times are longer because of the presence of late-eluting ECD-active interferences.

The two-step clean-up procedure generally used involves a liquid–liquid extraction with dichloromethane, and solid–liquid sorption using a Sep Pak C18 cartridge. PCP recovery over the 0.2–10 ppm range is 75–100%.

Several wood samples containing 1–50 ppm of PCP have successfully been analyzed, and the good potential of NPLC-ECD for trace-level determination of PCP has been demonstrated.

INTRODUCTION

Pentachlorophenol (PCP) is an inexpensive fungicide, bactericide, herbicide, molluscicide, algicide and insecticide that is widely used as wood preservative. It is usually applied as the technical product and is then contaminated with lower chlorinated phenols and other toxic chlorinated compounds.[1,2] PCP has pronounced acute toxicity to all forms of life, including man and its use is, therefore, strictly regulated. The presence of high concentrations of PCP residues on wood may lead to serious contamination of surface water and soil samples as well as food or inner house air. Food contamination occurs when PCP-containing woodshavings or paper are used as packing materials for crates, filled with foodstuffs.

Analytical procedures for PCP and other highly chlorinated phenols nowadays usually involve capillary gas chromatography (GC). In The Netherlands, for residue analysis the method of Krijgsman and Van der Kamp is usually employed.[3,4] This involves acetylation of the phenolic group with acetic anhydride, followed by GC with electron-capture detection (GC-ECD). Derivatization is necessary to facilitate GC separation. Gas chromatography–mass spectrometry (GC-MS) is generally used for confirmation purposes. It is a drawback that, usually, similar sample preparation steps are employed for GC-ECD and GC-MS, so that these are not really independent analytical methods. Besides, GC-MS is an expensive technique and MS facilities are not always available.

In the present study, we have investigated the potential of high-performance liquid chromatography (HPLC) as an independent (confirmation) method for the determination of PCP in wood samples. In the literature, various HPLC procedures for the determination of PCP have been published; these are reviewed in Tesarová and Pacáková.[5] For the analysis of wood samples, only a single procedure has been published[6] which involves reversed-phase HPLC on a C18-bonded phase. In the quoted paper, relatively high concentrations of PCP of 30–90 $\mu g/cm^2$ had to be determined, as the samples involved were surface-treated stored lumber; simple UV absorbance detection turned out to provide sufficient sensitivity and selectivity. We have tested four different HPLC procedures for the determination of PCP in wood samples, viz. reversed-phase HPLC with UV and amperometric detection (RPLC-UV and RPLC-AD, respectively) and normal-phase HPLC with UV and electron-capture detection (NPLC-UV and NPLC-ECD, respectively). Attention has mainly been devoted to aspects such as sensitivity and selectivity and the applicability to real samples.

EXPERIMENTAL

Materials

Sample pretreatment 2.5 g of wood shavings were extracted by overnight contact with 50 ml of acetone plus 2 ml of a 10% aqueous sulphuric acid solution. After filtration over cotton wool the residue was washed with acetone. The clean-up procedure consisted of three steps, A, B and C. Depending on the specificity of the method of analysis either step A, the combined steps A + B, or the extensive method, A + B + C, was used.

(A) To 20 ml of an acetone extract (corresponding to 0.5 g of wood), 5 ml of distilled water were added and the acetone layer was evaporated on a rotavapor. The water phase was extracted with 1×3 and 2×2 ml of dichloromethane. The dichloromethane extract was dried over sodium sulphate and, next, evaporated to dryness. The residue was dissolved in 2.5 ml of methanol for RPLC and for further clean-up, or in 1 ml of hexane-toluene (80:20) for NPLC.

(B) After the addition of 2.5 ml of water and 0.1 ml of 10%

sulphuric acid to the methanol solution, the sample was applied to a Sep Pak C18 (Millipore-Waters, Milford, MA, U.S.A.) cartridge. Elution took place with successively 25 ml of methanol-10% sulphuric acid (50:50) and 10 ml of methanol-10% sulphuric acid (70:30). The first 25 ml were discarded and the second fraction, which contains the PCP, was either used for direct injection into the RPLC system or further cleaned by extraction with petroleum ether. For NPLC 4 ml of 1% sulphuric acid were added to the solution and extraction was carried out with 2×5 ml of dichloromethane. The dichloromethane extract was dried over sodium sulphate and, next, evaporated to dryness. The residue was dissolved in 1 ml of hexane-toluene (80:20).

(C) Further clean-up of the second Sep Pak C18 fraction was carried out after the addition of 10 ml of water plus 0.2 ml of 10% sulphuric acid, using an extraction with 3×6 ml of petroleum ether. The combined organic extracts were dried over sodium sulphate and evaporated to dryness in a Kuderna-Danish apparatus. For RPLC the residue was dissolved in 1 ml of methanol-water (50:50) and aliquots were used for injection into the liquid chromatograph. For NPLC the dried petroleum ether extract was dissolved in 1 ml of hexane-toluene (80:20).

HPLC procedures

Chromatography RPLC was done on a 150×4.6 mm I.D. 5-μm Hypersil ODS column with methanol-0.01 M phosphoric acid (80:20) as the eluent, at a flow-rate of 1 ml min^{-1}. When amperometric detection was applied, sodium nitrate was added to the eluent at a final concentration of 0.4 g l^{-1}. NPLC was carried out using a 250×4.6 mm I.D. 10-μm CP Spher Silica (Chrompack, Middelburg, The Netherlands) column, with hexane-toluene-glacial acetic acid (80:19:1) or toluene-glacial acetic acid (99.8:0.2) as the eluent when ECD detection was used, and hexane-glacial acetic acid (99:1) when a UV absorbance detector was employed. The eluent flow-rate was 1 ml min^{-1} in all cases.

Equipment RPLC with amperometric and UV detection was carried out with a liquid chromatograph consisting of a Kipp (Delft, The Netherlands) Model 9208 pump, a Rheodyne (Berkeley, CA, U.S.A.)

Model 7520 injection valve with a 20-μl loop and a Perkin Elmer (Norwalk, CT, U.S.A.) Model BAS4D amperometric detector set at +1.0 V. For UV detection a Pye Unicam (Philips, Eindhoven, The Netherlands) LC-3 detector, set at 220, 230 or 254 nm, was used. Signals were recorded on a Kipp BD40 recorder.

For NPLC-ECD the HPLC system consisted of an Orlita (Giessen, F.R.G.) Model 034 sRC reciprocating pump, and a Valco (Houston, TX, U.S.A.) six-port injection valve with a 25-μl loop. A Valco tee-piece was used to direct a suitable portion (30–50%) of the effluent to the ECD, thereby obtaining optimum detector performance. The HPLC effluent was led to a Pye Unicam ^{63}Ni constant-current electron-capture detector via an evaporation interface. The interface essentially consists[7] of a 75 cm × 0.25 mm I.D. stainless-steel capillary enclosed in a massive stainless-steel block and kept at a temperature of 300°C. The interface was installed in the injection port of a Pye Unicam Model GCV gas chromatograph and directly connected with the detector via a stainless-steel capillary in the GC oven, which was also kept at a temperature of 300°C. For UV detection a Pye Unicam LC-3 detector, set at 220, 230 or 254 nm, was used.

RESULTS AND DISCUSSION

Reversed-phase liquid chromatography

RPLC-UV RPLC of pentachlorophenol with UV absorbance detection at 220–230 or 254 nm is a well-known procedure.[4,5] It is an attractive method because of its simplicity and reliability, and the repeatability using standard solutions in our work was excellent with a rel. S.D. of 1% (n=5) for 2–5 ng injections. The linear dynamic range was at least 3 orders of magnitude, with a detection limit of about 0.5 ng. Unfortunately, the potential of RPLC-UV at the wavelengths mentioned is seriously limited by the low selectivity, and trace-level analysis in complex matrices often creates problems.

The above assessment turned out to be true for the analysis of wood samples. In order to get useful results, the time-consuming clean-up method, A+B+C, had to be utilized. After this treatment, oak wood samples were relatively clean; extracts of pine wood samples, however, still showed a rather complex HPLC chromato-

gram and an "unknown" compound showed up at the PCP position ($t_R = 6.5$ min). Wavelength ratioing did not exclude the possibility that (approx. 0.2 ppm) PCP was indeed present in the samples analyzed. The results of the RPLC-AD work (cf. below), however, unequivocally showed this not to be true.

Our general experience indicates that RPLC-UV is only a useful technique when rather high concentrations of at least 5–10 ppm of PCP have to be determined.

RPLC-AD Amperometric detection, using oxidative voltammetry at glassy carbon electrodes, is known to be a selective detection mode for phenols.[8] Unfortunately, wood samples contain an abundance of phenolic compounds and some of those interfere with PCP determination.

In RPLC-AD, oak wood chromatograms always showed an "unknown" peak at the PCP position, corresponding to about 0.1 ppm PCP. As this peak was not observed in RPLC-UV the presence of PCP, at the said level, was excluded. A somewhat similar situation was encountered in the case of pine wood samples. Here, a peak showing partial overlap with PCP was detected amperometrically in extracts pretreated by the A+B+C method. Baseline resolution of the interfering compound and PCP was obtained by using a highly efficient column, indicating that the peak in the RPLC-UV pine extract chromatogram (cf. above) should not be assigned to PCP.

Obviously, RPLC-AD is applicable only when PCP concentrations of at least 2–5 ppm have to be determined in wood samples. Besides, even after the extensive A+B+C clean up, several matrix impurities still remain and cause an increase of the analysis time from about 7 min (PCP) to 15 min (last eluting compound; oak wood) or even 30 min (last eluting compound; pine samples).

Finally, it is well known that fouling of solid-state electrodes often occurs in RPLC after the repetitive injection of complex samples. In the present work, good repeatability was observed (rel. S.D. 3%; $n = 10$) upon the injection of suitably pretreated oak wood samples spiked at the 1-ppm level (10 ng PCP per injection). For quantitation, external standardization was, therefore, used after each five sample analyses. Over the tested range of 0.5–50 ng PCP per injection, the calibration curve had a correlation coefficient of 0.9992 (10 data points).

Normal-phase liquid chromatography

NPLC-UV In NPLC with UV detection at 230 nm the selectivity towards PCP in wood samples was slightly better than in RPLC-UV. However, due to baseline noise the detection limit for PCP, under the chromatographic conditions used, was 5 ng and the minimum detectable concentration in a wood extract pretreated by the A+B+C procedure was 1 ppm. In view of the significantly deteriorated sensitivity of NPLC-UV compared to RPLC-UV, the former technique was not studied in more detail.

NPLC-ECD Electron-capture detection has successfully been applied by us for the HPLC analysis of PCP and other chlorinated phenols.[9] Although RPLC can also be combined with on-line ECD, a narrow-bore system has to be used in this case and the sensitivity, in units of concentration, is about one order of magnitude less than with NPLC,[10] which can be used in combination with ECD at the normal-bore scale.

For the above reason, we preferred to work with a NPLC-ECD system using a silica column. For optimal separation between tri-, tetra- and pentachlorophenols an eluent composition of hexane-toluene-glacial acetic acid (80:19:1) was used. Addition of acid to the eluent was necessary in order to obtain sharp peaks. The resulting chromatogram is shown in Figure 1. For PCP standards, the minimum detectable amount with this system was 40 pg (signal/noise = 3:1), which is at least one order of magnitude better than with RPLC-UV. The linearity of NPLC-ECD was excellent with a correlation coefficient of 0.9999 (8 data points) for 0.04–25 ng PCP. The repeatability of the PCP determination in standard solutions showed a rel. S.D. of 1.2% ($n=4$) for a 9 ng injection.

The selectivity of electron-capture detection should be better than that of UV or electrochemical detection, since wood samples are expected to contain relatively few electron-captive compounds. This was indeed found to be the case, as is demonstrated in Figure 2. In this figure, chromatograms are shown of non-spiked pine and oak wood extracts, and of extracts spiked with 0.2 and 0.5 ppm PCP. The clean-up procedure used was method A+B. The peak at 6.3 min in the non-spiked sample probably represents a trace-level amount (0.04 ppm) of PCP. For these analyses toluene-glacial acetic acid (99.8:0.2) was used as the eluent to decrease analysis time. With this

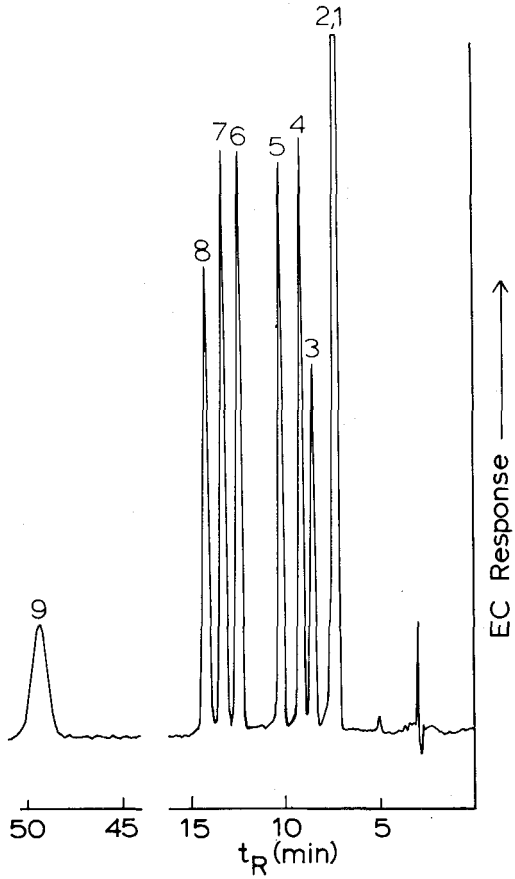

FIGURE 1 Separation of tri-, tetra- and pentachlorophenols in the NPLC system CP Spher silica/hexane-toluene-glacial acetic acid (80:19:1), using electron-capture detection. (1) 2,3,6-tri-, (2) 2,3,5,6-tetra-, (3) 2,3,4,6-tetra-, (4) penta-, (5) 2,3,5-tri-, (6) 2,4,5-tri-, (7) 2,3,4-tri-, (8) 2,3,4,5-tetra-, (9) 3,4,5-trichlorophenol. Further conditions, see Experimental.

clean-up method, the limit of determination of PCP in wood samples is approx. 4 ppb (50 pg per injection). The rel. S.D. for PCP determination at the 0.5 ppm spiking level (6 ng PCP per injection) was 4% ($n=3$).

The analysis of wood samples after clean-up with method A only, and using hexane-toluene-glacial acetic acid (80:19:1) as eluent, in

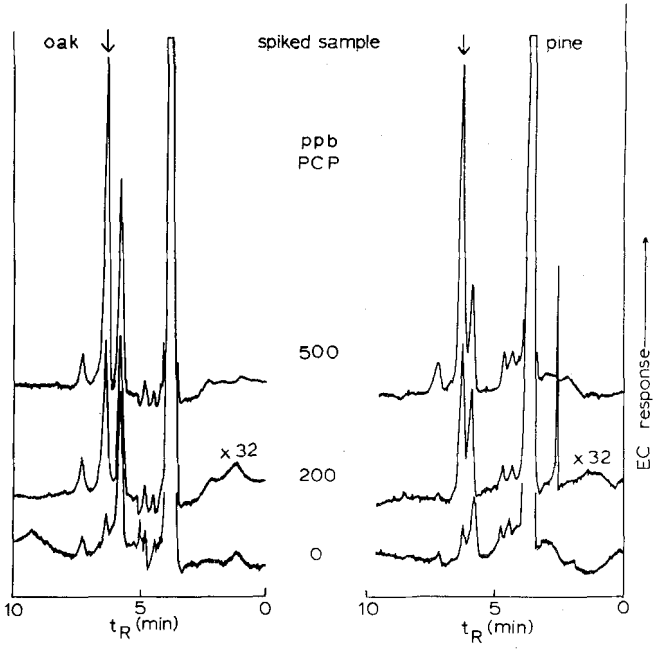

FIGURE 2 NPLC-ECD determination of PCP in a pine and oak wood extract after clean-up by procedure A+B in the system CP Spher silica/toluene-glacial acetic acid (99.8:0.2). Spiking level (from top to bottom): 0.5 ppm, 0.2 ppm, no spike added. ECD: detector current, 1.10^{-10} A; attenuation, ×32. Volume injected, 25 µl. Further conditions, see Experimental.

principle was also possible. However, analysis frequently took a rather long time (25 min) because of the presence of late-eluting ECD-sensitive compounds; this was especially observed with oak samples. The repeatability of the procedure involving clean-up step A only was satisfactory down to a spiking level of 0.1–0.2 ppm, with a rel. S.D. of about 6% ($n=3$).

Recovery studies

The recovery of PCP from spiked wood samples, using either sample preparation method A+B or method A+B+C, was determined at different spiking levels. All measurements were done with RPLC-AD

except those at a spiking level of 0.05–0.2 ppm using method A+B, which were done with NPLC-ECD. The results are given in Table I. It is evident that step C (petroleum ether extraction of the Sep Pak eluate) causes rather large losses at all concentration levels. This step therefore should preferably be omitted. Method A+B gives satisfactory results of 75–100% recovery for PCP concentrations in the 0.2–10 ppm range. At still lower spiking levels of 0.05–0.1 ppm the recovery with method A+B was found to decrease to 60±10%. At these low levels the recovery with clean-up via method A only and using NPLC-ECD, was determined to be 75±5% ($n=3$).

TABLE I

Recovery of PCP from spiked wood sample extracts, after clean-up with methods A+B and A+B+C.[a]

Spiking level (ppm)	% recovery±rel. S.D. for:	
	A+B	A+B+C
0.05	60±10	—
0.1	60±10	39±15
0.2	80±9	—
0.5	76±3	60±12
1.0	102±2	66±5
5.0	82±2	63±5
10.0	90±0.5	65±3

[a]All data are the mean of 4 independent sample analyses.

In the experiments at a 0.5 ppm PCP level (method A+B), the final determination was done by both RPLC-AD and NPLC-ECD. The same result was obtained in both cases, which demonstrates that the additional extraction step necessary to prepare a solution of PCP in hexane-toluene for NPLC after the Sep Pak clean-up (see section Experimental) does not result in an extra loss of PCP.

Sample analysis

In order to demonstrate the applicability of the various analytical methods we analyzed several wood samples. As a first application,

extracts of two wood samples known to contain relatively high PCP concentrations were measured. The analyses by means of RPLC-UV, RPLC-AD and NPLC-ECD mutually showed excellent agreement with PCP levels of 30, 29 and 28 ppm for one, and 51, 49 and 48 for the other sample.

Finally, two wood samples were analyzed which were known, from reference GC analysis, to contain in the order of a few ppm of PCP. Figure 3 shows chromatograms of one wood extract, after clean-up using method A+B+C, and analysis by NPLC-ECD (3a) and NPLC-UV (3b); with the former technique the sample was 4-fold diluted before injection. The better selectivity and sensitivity achieved with ECD compared to UV detection can clearly be seen

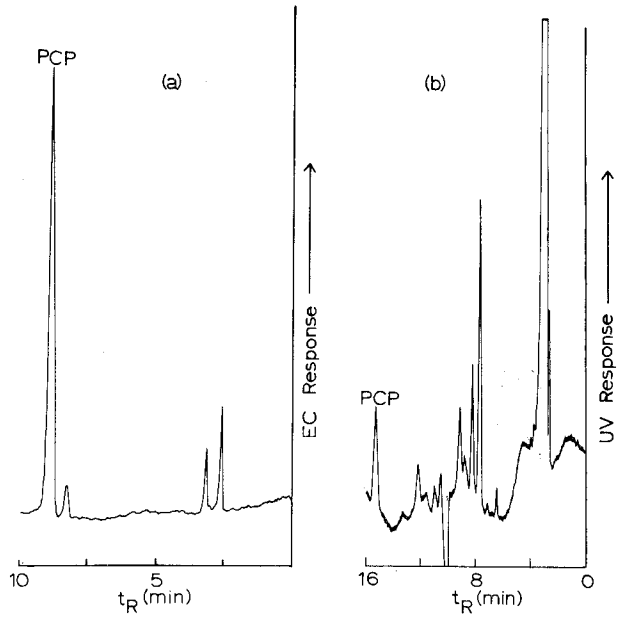

FIGURE 3 Analysis of a wood sample extract, after clean-up by procedure A+B+C, using NPLC-ECD (a) and NPLC-UV (b). For the NPLC-ECD analysis the system CP Spher silica/hexane-toluene-glacial acetic acid (80:19:1) was used and the sample was 4-fold diluted before injection. For the NPLC-UV analysis the system CP Spher silica/hexane-glacial acetic acid (99:1) was used. ECD: detector current, 1.10^{-10} A; attenuation, × 32. UV: detection at 230 nm; AUFS, 0.02. Volumes injected, 25 μl. Further conditions, see Experimental.

from this figure. The PCP concentration was determined to be 3.6 and 7.0 ppm, with NPLC-ECD and NPLC-UV, respectively. The small peak visible in Figure 3a at 8.3 min has the same retention time as 2,3,4,6-tetrachlorophenol, which is the major impurity (4–8%) in the commonly technical-grade PCP used in the wood-treatment industry.[11] The wood extract was also measured with RPLC-AD and RPLC-UV. The PCP levels found were 2.4 and 4.0 ppm, respectively.

Figure 4 shows chromatograms of the other wood extract, also after clean-up using method A+B+C, and analysis by NPLC-ECD (4a), RPLC-UV (4b) and RPLC-AD (4c). The PCP levels found were 0.2, 0.4 and 1.6 ppm, respectively. Figure 4 clearly shows the lack of selectivity of both RPLC-AD and RPLC-UV. At this PCP level the

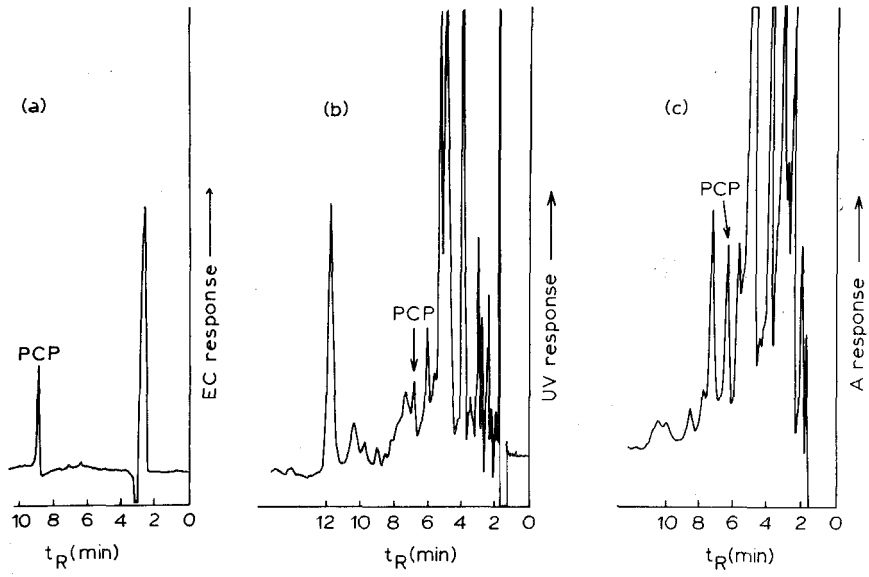

FIGURE 4 Analysis of a wood sample extract, after clean-up by procedure A+B+C, using NPLC-ECD (a), RPLC-UV (b) and RPLC-AD (c). Conditions for NPLC-ECD as in Figure 3. For the RPLC-UV analysis the system Hypersil-ODS/methanol-0.01 M phosphoric acid (80:20) was used. For the RPLC-AD analysis sodium nitrate was added to the same eluent at a final concentration of 0.4 $g\,l^{-1}$. UV: detection at 220 nm; AUFS, 0.01. AD: 5 nA full scale. Volumes injected, 20 μl. Further conditions, see Experimental.

NPLC-ECD analysis was slightly hampered by a dip occurring in the chromatogram just before the PCP peak. It is evident that with the extracts containing lower levels of PCP the mutual agreement between the various results certainly will not be satisfactory.

It is interesting to add that in the above cases relatively low PCP concentration levels are calculated when using the analytical technique which combines high selectivity and sensitivity, i.e. NPLC-ECD.

CONCLUSIONS

If concentrations of PCP of over 1 ppm are present in wood samples, RPLC using either amperometric or UV detection, and clean-up according to procedure A+B+C can be used. Since there are no significant over-all differences in selectivity between RPLC-UV and RPLC-AD, the more reliable and easy to operate UV detector will be preferred. The higher sensitivity of the amperometric detector is not a relevant aspect here because of its insufficient specificity in the case of wood samples. The extensive clean-up procedure A+B+C, however, causes low and irreproducible recoveries at PCP levels of less than about 1 ppm and its usefulness therefore is limited.

For the analysis of both low and high concentrations of PCP in wood samples, NPLC with electron-capture detection offers excellent selectivity and sensitivity. Even low ppb levels of PCP can now be determined, and linearity and reproducibility are satisfactory. The NPLC-ECD procedure involves clean-up of the wood extracts by liquid–liquid extraction and sorption/elution using a Sep Pak C18 cartridge, and shows good recoveries of 75–100% over the 0.2–10 ppm range. In principle the Sep Pak clean-up step can be omitted; however, this leads to longer analysis times because of the presence of late-eluting ECD-sensitive compounds in the wood sample extracts. For a summary of the results, one is referred to Table II.

The results obtained with the various analytical methods for some real wood samples with PCP concentrations of 20–50 ppm are mutually in good agreement. However, at PCP concentrations of about 1 ppm the results obtained with the different procedures start to show considerable mutual divergence. Even though this aspect requires further attention, it seems safe to conclude that NPLC-ECD

TABLE II

Comparison of LC methods[a] used for the determination of PCP in wood samples.

LC \ Detection	UV	AD	ECD
NPLC	A+B+C high ppm	—	A+B(+C) >0.1–0.2 ppm
RPLC	A+B+C >5–10 ppm	A+B+C >2–5 ppm	—

[a]For experimental details, see text; A, B, C: clean-up steps.

is the recommended method because of its good selectivity and excellent sensitivity towards PCP. In a subsequent study we shall devote our attention to a comparison of GC-ECD and NPLC-ECD, especially for samples with PCP concentrations in the ppb range.[12]

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