SEPARATION OF SOME HALOGENATED POLLUTANTS BY HPLC ON AN AMINOPROPYLSILICA STATIONARY PHASE

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Analysis of halogenated pollutants in environmental samples is mostly performed by gas chromatography with electron capture detector (ECD) for sensitive and selective detection. This procedure is complicated by the number of compounds, the concentration differences between compound, and interfering compounds. With some types of samples negative peaks occur in the chromatograms, adding to the complexity. This problem has especially been observed in connection with the analysis of pine needle extracts with respect to polychlorinated biphenyls (PCB). To overcome the problem with interfering compounds and negative peaks a method based on liquid chromatography utilizing an aminopropylsilica column in straight phase mode was developed. The column was further characterised using a number of groups of halogenated environmental pollutants, such as polychlorinated naphtalenes (PCN), chlorinated paraffins (CP), a number of chlorinated pesticides and polybrominated diphenyl ethers (PBDE).

EPA reference materials were used as standards for pesticides. Individual PCB congeners were synthezised at the Environmental Chemistry Department according to [1], and no 189 was used as internal standard. Technical PCB products were Aroclors 1248, 1254 and 1260, (Monsanto, USA) and Clophens A50 and A60 (Bayer, Germany). Polychlorinated naphtalenes were the technical mixture Halowax 1013 (Koppers, USA), and CPs were Hüls 40 and 70 (Hüls, Germany). The tested PBDEs were 2,2',4,4'-tetra-BDE and the technical mixtures Bromkal 70-5 DE and 79-8 DE (penta- and octa-BDE respectively, Chemische Fabrik Kalk, Germany) and deca-BDE (FR-300BA, Dow, USA).

Fractionation was done using a Waters 590 pump and a Shimadzu SPD-2AS UV-detector operated at 254 nm, utilizing a Waters 300 mm x 3.9 mm 5 µm µ-Bondapak aminopropylsilica column with hexane as mobile phase. The fractions were analysed on a Varian 3400 gas chomatograph equipped with an ECD. The injection port was held at 250°C, and the detector at 360°C with nitrogen as makeup gas. The column was a DB-5 (30 m x 0,25 mm, J&W Scientific, USA) programmed as follows, 80° for 2 min, 10°/min

to 280°, final temperature held for 10 min. Hydrogen was used as carrier gas.

Pine needles were taken fresh from the trees immersed in dichloromethane for 48 hours. Wax extracts were filtered and worked up using silica gel with 33% concentrated sulphuric acid and hexane/benzene 1:1 as eluting solvent. An in depth description of the work-up procedure is given in Jensen et al. [2].

The aminopropylsilica column in straight phase mode has been used to separate hydrocarbons according to the number of fused aromatic rings. Thus aliphatic and olefinic compounds elute prior to aromatic, and the aromatic compounds can be separated according to the number of fused aromatic rings [3]. The mechanism behind the separation is supposedly an electron donor/acceptor complex forming between the π-electrons in the analyte and the lone pair electrons in the aminogroup [4]. The incentive for starting this work, was to apply this type of separation to halogenated environmental pollutants to separate chlorinated aromatics from the chlorinated aliphatics, to get fractions that can be analysed directly by GC/ECD with a minimum amount of interferences.

In order to isolate a PCB fraction the samples were divided into three fractions. A pre-fraction consisting of compounds eluting prior to the start of the elution of the technical PCB product A60. The PCB fraction was obtained by heart cutting between this point and the last eluting PCB congener, IUPAC no 128. At this point the flow of the column was reversed and a fraction with the remaining material was eluted as one peak. An example of an HPLC chromatogram of a real sample is shown in figure 1.

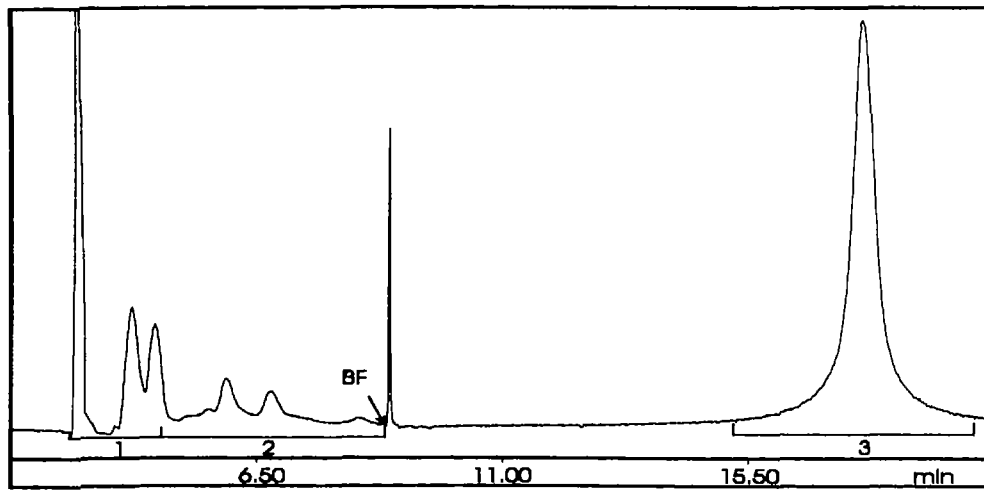

Figure 1: HPLC clean-up of pine needle wax extract. BF = back-flush point, 1 = pre-fraction, 2 = PCB fraction, 3 = back-flush fraction.

The method was applied for the analysis of PCBs in extracts of pine needle wax cleaned up for analysis of chlorinated pollutants according to the procedure described by Jensen et al. [2]. In these extracts it was very difficult to identify and quantify any PCBs

(figure 2). After clean-up using the HPLC method, most peaks in the ECD trace of the extract was shown to correspond to PCB congeners in a Clophen A50 standard. The interfering components were reduced to p,p-DDE and four unidentified components (figure 3). Most of the interfering compounds as well as the components generating the negative peaks in the GC/ECD analysis were thus removed enabling both identification and quantitation of peaks in the obtained chromatograms.

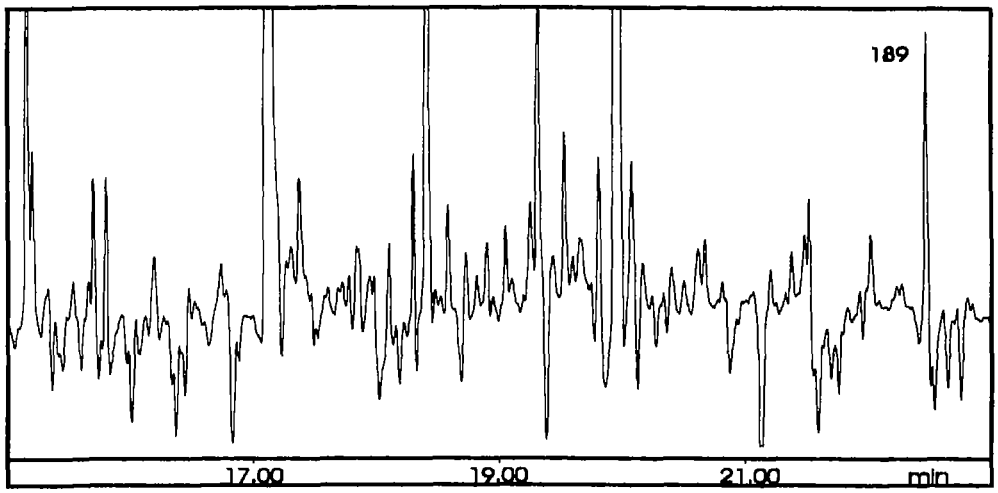

Figure 2: GC/ECD chromatogram from PCB analysis of pine needle wax extract prior to HPLC clean-up. 189 = PCB congener IUPAC 189 which was used as internal standard.

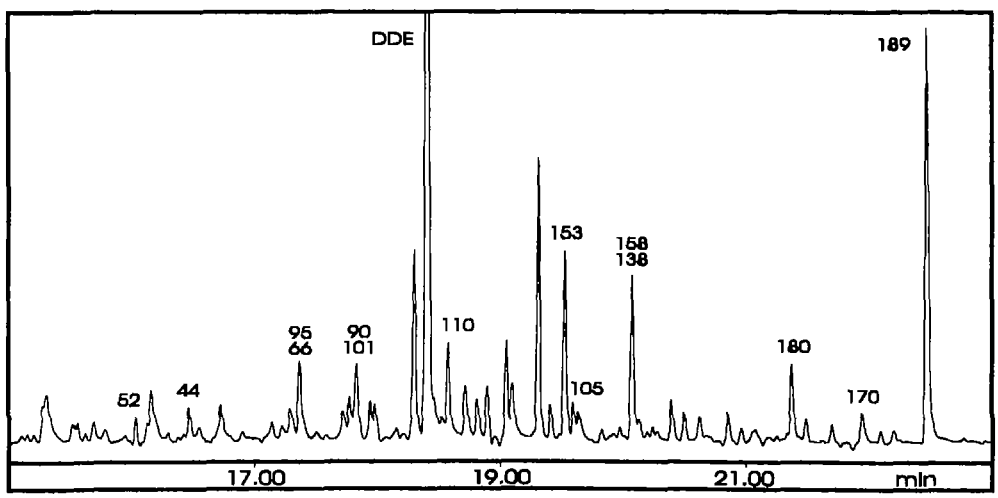

Figure 3: GC/ECD chromatogram from PCB analysis of pine needle wax extract after HPLC clean-up. Numbers are designating some of the PCB congeners according to the IUPAC enumeration.

The fractions were further characterized with respect to their contents of a number of groups of halogenated environmental pollutants. A number of interesting observations were made. It was found that PCBs and chloroparaffins could be completely separated, but, contrary to aliphatic hydrocarbons, the CPs eluted after the dicyclic aromatic PCBs and PCNs. Another interesting observation was that all tested polybrominated diphenyl ethers were separated from the PCB fraction and eluted in the pre-fraction. Table 1 gives a summary of results from the characterization of the fractions. Recovery tests of the HPLC fractionation indicates a recovery of 90-100% for the tested compounds.

FRACTION 1	FRACTION 2	FRACTION 3
Hexachlorobenzene	PCB	CP
PCN 33%	PCN 67%	α- β- γ- & δ -HCH
Mirex	Toxaphene 5%	Toxaphene 95%
Decachlorobiphenyl	p,p-DDE	p,p-DDT & p,p-DDD
PBDE	Heptachlor	p,p-metoxychlor
	α- & γ-chlordene	Dieldrin & Endrin
	Chlordene	Oxychlordane
		α- & γ-chlordane
		Transnonachlor

Table 1: Characterization of the fractions from the aminopropylsilica column with respect to the different halogenated compounds tested. PCB - all polychlorinated biphenyls except decachloro biphenyl; CP - chlorinated paraffins Hüls 40 and 70; PCN - Halowax 1013; HCH - hexachlorocyclohexane; PBDE - polybrominated diphcylethers

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