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# Isolation of chlorinated dibenzothiophenes by high-performance thin-layer chromatography

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## ABSTRACT

Chlorinated dibenzothiophenes can cause environmental problems analogous to chlorinated dibenzodioxins and dibenzofurans. In the analysis of chlorinated dibenzodioxins by gas chromatography–mass spectrometry some unknown chlorinated compounds have been found. The chlorinated dibenzothiophenes have the same $m/z$ values as the corresponding dioxins in low-resolution mass spectrometry and they are found in same planar aromatic compound fraction in alumina and carbon column chromatography. Some chlorinated dibenzothiophenes were synthesized to serve as model compounds in analytical work. High-performance thin-layer chromatography (TLC) with different plates and several eluents was tried to find a separation method for the chlorinated dibenzothiophenes. The experiments showed that reversed-phase TLC with RP-18 plates and acetonitrile–water eluents could separate the chlorinated dibenzothiophenes from many interfering compounds but not completely from complex environmental samples.

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## INTRODUCTION

Chlorinated aromatic compounds are present as harmful compounds in the environment. The chlorine derives mainly from anthropogenic activities and is incorporated in the planar aromatic compounds (PAC) structures during combustion processes. Chlorinated dibenzothiophenes can cause environmental problems analogous to chlorinated dibenzodioxins and dibenzofurans. In analysing chlorinated dibenzodioxins and dibenzofurans by gas chromatography–mass spectrometry (GC–MS) some unknown chlorinated compounds have been found which may be planar aromatic sulphur compounds. Chlorinated dibenzothiophenes in the purification and fractionation process that we use are found in the same fraction as the corresponding dioxins and dibenzofurans, hence these compounds may represent some of the unknown compounds in this fraction [1,2]. The chlorinated dibenzothiophenes have the same $m/z$ values as the corresponding dioxins in high-resolution GC–low-resolution MS. A resolution of about 20 000 is needed to separate, for example, tetrachlorodibenzothiophenes (MW = 319.87880) from tetrachlorodioxins (MW = 319.89650).

The separation of the polyaromatic sulphur- and chlorine-containing compounds from interfering compounds in GC–MS and in GC, although a flame photometric detector in the sulphur mode was used, has been found to be difficult. Different liquid chromatographic fractionations have to be applied to obtain the

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required fraction with minimum amounts of interfering compounds [3].

In environmental samples, dibenzothiophenes usually occur in a complex mixture of organic compounds, so the first step is to separate the non-polar aromatic compound fraction which contains many kinds of alkylated and chlorinated compounds. This is usually done by silica gel or alumina column chromatography using hexane and hexane–dichloromethane as eluents. There is no unambigious method for separating the sulphur- and/or chlorine-containing compounds from other aromatic compounds in this fraction [3].

The complexation of organic sulphur compounds by means of copper, mercury, palladium and other metal salts has been used to separate the former. $PdCl_2$ is a good complexing agent for aromatic sulphur heterocycles, but further analysis of the palladium–sulphur compound complexes was found to be impractical owing to difficult degradation (the complex cannot be analysed by GC, GC–MS or high-performance liquid chromatography) [3–5].

In the mass spectrometric analysis of chlorinated and methylated aromatic sulphur compounds, the alumina or carbon column purified PAC fraction from environmental samples still contains many interfering compounds because an electron impact mass spectrometer is not sufficiently selective for compounds with a high electron affinity or sulphur to be able to confirm these compounds as chlorinated dibenzothiophenes. The concentrations of these compounds, if present in environmental samples, are very low compared with the other compounds in the same fraction, so better separation methods are needed.

## EXPERIMENTAL

### *Compounds and samples*

Several methylated and chlorinated dibenzothiophenes were synthesized for use as model compounds in the development of separation methods and in environmental and toxicological analysis of these compounds. Some of the lower isomers were obtained as pure compounds but mainly the synthetic products were mixtures of several different isomers, mainly with the same degree of chlorination or methylation.

The following compounds were prepared: 2-, 3- and 4-chlorobiphenyl, 2,4,5-trichlorobiphenyl, a mixture of 2,3,3′,4- and 3,3′,4,4′-tetrachlorobiphenyl, 2,4,5-trichlorodibenzothiophene and mixtures that contained several trichlorinated and tetrachlorynated biphenyls and dibenzothiophenes. The preparation of the model compounds is described elsewhere [2]. Pure dibenzothiophene was purchased from Fluka and dibenzothiophene 5,5-dioxide (sulphone) was prepared from this by oxidation.

Different non-polar aromatic compounds and planar aromatic compound fractions were obtained from environmental samples [1,6]. The non-polar aromatic compound fractions were originally prepared from environmental samples such as sediments, fish, mussel and oils for the analysis of polycyclic aromatic hydrocarbons and some non-polar aromatic oil residue compounds and the planar aromatic compound fractions from biological samples for the analysis of dioxins and dibenzofurans.

TABLE I

TLC PLATES AND ELUENTS USED

| TLC plate | Eluents |
|---|---|
| *Normal-phase* | |
| Aluminiumoxid | Hexane, dichloromethane, light |
| Kieselgel | petroleum, toluene, acetone |
| $NH_2$ | |
| *Reversed-phase* | |
| $NH_2$ | Acetonitrile–water, methanol–water, ethanol–water |
| RP-18 | (100:0, 90:10, 80:20 and 70:30) |

### *Thin-layer chromatography*

Chemicals were spotted with the aid of microsyringes on high-performance TLC plates. Chromatograms were obtained by ascending elution with mixtures of different solvents in closed tanks after equilibration with the aid of wet filter-paper. Aromatic UV-quenching substances were detected as dark or violet spots on fluorescent plates on illumination with UV radiation at 254 nm.

The following plates were used: Kieselgel 60 $F_{254}$ (10 × 20 cm, thickness 0.25 mm, Merck, Art. 11846), Aluminiumoxid 60 $F_{254}$ (20 × 20 cm, thickness 0.25 mm, Merck, Art. 5713), $NH_2F_{254}$ für die Nano-DC (10 × 10 cm, thickness 0.2 mm. Merck, Art. 15647) and RP-18 $F_{254}S$ (5 × 20 cm, thickness 0.25 mm Merck, Art. 15683).

In normal-phase chromatography, with Aluminiumoxid, Kieselgel and $NH_2$ plates were used with several organic solvents and their mixtures. In reversed-phase chromatography, $NH_2$ and RP-18 plates were tried with different acetonitrile–water, ethanol–water and methanol–water mixtures (Table I).

## RESULTS AND DISCUSSION

The $NH_2$ TLC plates were used in both the reversed- and normal-phase modes. In reversed-phase TLC different mixtures of methanol–water, acetonitrile–water and ethanol–water were tried. In normal-phase TLC organic solvents such as hexane, dichloromethane, light petroleum and toluene were used.

In RP-$NH_2$ TLC no separation could be achieved with methanol–water mixtures from methanol–water (1:1) to pure methanol. Oxidized sulphur compounds such as dibenzothiophene 5,5-dioxide were not eluted at all and other aromatic compounds, irrespective of the sulphur or chlorine content, had the same relative retention times, increasing nearly linearly with the increasing methanol concentration in the eluent. With ethanol–water eluents the relative retention times were higher but the separation of the components studied was not improved. Acetonitrile–water eluents could not be used to elute the $NH_2$ TLC plates as they did not ascend linearly on the plates.

In normal phase $NH_2$ TLC with hexane the chlorinated biphenyls and dibenzothiophenes were found to elute faster than the corresponding non-chlorinated compounds. However, the compounds could not be separated completely into these two groups. Dichloromethane eluted all the chlorinated and non-chlorinated com-

TABLE II

RP-18 TLC RESULTS ($R_F$ VALUES) WITH ACETONITRILE–WATER ELUENTS

| Acetonitrile–water | Sulphones | 1–3-PAH | Cl-BP | Cl-DBT |
|---|---|---|---|---|
| 100 | 0.64–0.68 | 0.57–0.62 | 0.43–0.49 | 0.48–0.54 |
| 90:10 | 0.64–0.69 | 0.52–0.58 | 0.35–0.40 | 0.42–0.49 |
| 80:20 | 0.55–0.64 | 0.38–0.48 | 0.18–0.26 | 0.28–0.35 |
| 70:30 | 0.46–0.54 | 0.27–0.34 | 0.11–0.16 | 0.21–0.26 |

pounds without any difference in retention times. Only oxidized compounds were more retained. Hexane–dichloromethane eluents did not improve the separation.

In reversed-phase TLC with RP-18 plates and acetonitrile–water eluents, the chlorinated dibenzothiophenes could be separated from chlorinated biphenyls, some oxidized dibenzothiophene derivatives and many other polycyclic aromatic compounds. Table II gives the results of RP-18 TLC experiments with acetonitrile–water for a mixture of chlorinated dibenzothiophenes (Cl-DBT), chlorinated bihenyls (Cl-BP), some low-molecular-weight aromatics without sulphur or chlorine (1–3-PAH) and some oxidized dibenzothiophenes (sulphones). With methanol–water eluents no separation could be achieved.

RP-18 TLC can be used to isolate the chlorinated dibenzothiophenes from the synthetic mixtures or from some relative simple environmental samples (some air or water samples), but not from fractions from complex polluted biological samples that contain hundreds of different aromatic compounds, many of which contain chlorine or sulphur. RP-18 TLC can be used apparently to decrease the number of compounds in these fractions. No separation of the examined compounds was achieved with alumina or silica plates.

## CONCLUSIONS

Individual polycyclic aromatic hydrocarbons can be separated and analysed by RP-TLC using methanol–acetonitrile–water eluents [7–11].

Dibenzothiophenes as a separate group cannot be separated from the other compounds in the non-polar aromatic compound fraction using RP-TLC, but when oxidized to the corresponding sulphones to increase their polarity the separation can be achieved. Subsequent quantitative analysis must be done using high-performance liquid chromatography or GC–MS with a polar column [12]. However the sulphones are highly polar non-volatile and may be thermolabile, and hence cannot easily be analysed by GC.

The PAC fractions extracted from environmental samples are very complex and contain many different kinds of alkylated and chlorinated aromatic non-polar compounds. It is not possible to isolate chlorinated dibenzothiophenes as a separate group from fractions produced by extracting complex environmental samples using only TLC. However, by using TLC we can apparently decrease the number of different compounds in the fraction containing the chlorinated dibenzothiophenes.

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