A COMPLETE METHOD FOR THE QUANTITATIVE ANALYSIS OF PLANAR, MONO, AND DIORTHO PCB'S, POLYCHLORINATED DIBENZO-DIOXINS, AND FURANS IN ENVIRONMENTAL SAMPLES

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Abstract

A novel method is presented for the separation and quantitative determination of planar (non-ortho), mono-ortho, di-ortho PCB's, polychlorinated dibenzo-dioxins (PCDD's) and Dibenzo-furans (PCDF's) in environmental samples. The procedure, which combines the advantages of acid-silica and alumina cleanup is then complemented by the separation, of the targeted analytes, by selective elution from an AX-21Activated Charcoal/ Silca gel cromatographic column based on the "relative planarity" of the molecules. The protocol allows for an independent estimate of the compounds responsible for more than 99% of the "dioxin-like" toxicity by analizing four different fractions using high resolution gas chromatography with electron capture detection and isotopic dilution high resolution gas chromatography/high resolution mass spectrometry. The procedure allows the selective determination of AHH active PCB's, Planar PCBs, Dioxins and Furans at the parts per tillion levels.

Introduction

There is considerable evidence that most of the toxicity observed in aquatic ecosystems is mainly due to the presence of PCDD's, PCDF's and other "Dioxin-Like" structures which includes the well known "Planar" or "non-ortho" PCB congeners 3,3',4,4-tetrachlorobiphenyl (IUPAC#77), 3,3',4,4',5-pentachlorobiphenyl (IUPAC# 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC#169) which are also strong AHH and EROD inducers.[1] These "planar" or "non-ortho" PCB congeners are present in lower concentrations than other PCB congeners such as PCB-118, PCB-105, PCB-138, PCB-128, PCB-156, PCB-157. However, these congeners can be responsible for much of the toxic equivalents in environmental samples.[2,3] Although the determination of these PCBs is crucial for environmental studies, their analysis is usually complicated by two factors: 1) the relative low abundance of the "toxic"congeners compared to the bulk of the PCBs and 2) their co-elution with other non targeted compounds on most GC columns.[4] Even tough several selective enrichment methods have been developed, most of them are employed for the analysis of the four toxic "non-ortho" congeners (PCB-77, PCB-81,PCB-126 and PCB-169).[5,6,7,8]

The present paper reports an analytical protocol for the separation and determination of the non-ortho, mono-ortho and di-orthoPCB's, as well as Dioxins and Furans in the same environmental sample.

EXPERIMENTAL

Glassware, solvents and chemicals were pesticide grade or cleaned and conditioned as usual for trace organic analysis. All the native target analytes were obtained from Ultra Scientific (Kingstown, Rhode Island, U.S.A.) while all labeled compounds were obtained as concentrated solutions from Cambridge Isotope Laboratories (Andover, Massachusetts, U.S.A.)

Sample Preparation and Cleanup

To test the analytical protocol, commercially available eggs were spiked with the appropriate internal and recovery standards and, as required, with a mixture of all the targeted analytes or a 1:1:1:1 mixture of Aroclor 1248, Aroclor 1254 and Aroclor 1260.
The samples (10g) were weighed into 200 ml centrifuge bottles and mixed with sodium sulfate (40 g). After adding 100 ml of methylene chloride, the samples were macerated by using a Tekmar tissuemizer (Cincinnati, Ohio, U.S.A.) for 5 min. Two more extractions with 100 ml of methylene chloride were performed and, after the sample was centrifuged, the solvent extracts were combined in a 500 ml flat bottom flask. After the extraction, the samples are concentrated and the solvent is exchanged to 100 ml of hexane. To eliminate interferences caused by lipid material, the hexane extract was treated with 40 g of a 44% mixture of concentrated sulfuric acid/silica gel (Silica Gel 60, EM Science, Gibbstown, New Jersey, U.S.A.) and the samples were shaken for 2h. After filtration and concentration, the extracts were ready for chromatographic cleanup.

Mixed-Bed Silica Column

The concentrated sample in hexane was further purified using a 13 mm ID x 300 mm length chromatography column containing from top to bottom: 1 cm of quartz sand, 1 cm of sodium sulfate, 2g of silica gel (Silica Gel 60, EM Science, Gibbstown, New Jersey, U.S.A.), 8g of 44% sulfuric acid /silica gel, 1g of silica gel, 4g of 33% 1N sodium hydroxide/silica gel, 1g of silica gel and a glass wool plug. After the column was pre-rinsed with hexane, and the sample loaded, the column was eluted with 120 ml of hexane. The collected eluate is then evaporated to 1 ml by using a rotary evaporator.

Basic Alumina Column Cleanup

The hexane concentrate obtained from the mixed-bed column is then applied to a 13 mm ID x 300 mm length chromatography column containing, from top to bottom: 1 cm of sodium sulfate, 6 g of alumina (Alumina, Activated, 80-200 mesh, EM Science, Gibbstown, New Jersey, U.S.A.). The column is then eluted first with 60 ml hexane and the eluate is discarded. The target analytes are then recovered by eluting the column with 40 ml of a 70:30 hexane-methylene chloride mixture. The collected eluate is then evaporated to 1 ml by using a rotary evaporator.

Charcoal Column Cleanup

All samples were fractionated using a low pressure Michael-Miller type chromatographic column (10 mm ID x 300 mm length, ACE Glass Inc., Vineland, New Jersey, U.S.A.) The glass column was fitted with removable Teflon threaded couplings, stopper, an adjustable flow valve and a 250 ml glass solvent reservoir . The column was packed by placing, form top to bottom, a glass wool plug, 1 cm of sand, 2 g of the charcoal/silica adsorbent (20:1 mixture of Silica Gel 60, EM Science, Gibbstown,

New Jersey, U.S.A.) and AX-21 Super Activated Carbon (Anderson Inc., Adrian, Michigan, U.S.A.); 1 cm of sand and another glass wool plug. After this step, the column was washed successively with 20 ml each, toluene, methylene chloride, and cyclohexane before the sample is applied. After the column is drained to the sand bed, the purified sample extract in 1 ml of hexane is added to the column. Three fractions are then eluted from the column in the forward direction. Fraction one (F1) containing the 2+Ortho PCB'S is recovered with 60 ml. of a 8:2 mixture of cyclohexane-methylene chloride; Fraction two (F2) containing the Mono-Ortho PCB's is then recovered with 40 ml. of a 9:1 mixture of methylene chloride-toluene and finally, Fraction 3 (F3) which contains the more toxic Planar PCB's is eluted with 25 ml. of toluene. After this fraction is collected, the column is "Flipped" and Fraction 4 (F4), containing the PCDD's/PCDF's is collected by eluting the column backwards with 200 ml of toluene. Depending on the internal standards used, these fractions can then be analized by using either HRGC/ECD or HRGC/HRMS techniques.

Results and Discussion

Since the analysis of the Aroclor mixtures is complicated a simple test sample was used to validate the experiments. The sample, spiked with all the toxic PCB's and selected PCDD isomers was analyzed to evaluate the initial column separation. The results, presented in Figure 1 shows that the charcoal chromatography allowed a successful determination of all the "toxic" PCB's, PCDD's and PCDF's. The figure, showing the composition of the original spike on top and the successive separation of the targeted analytes according to their increasing planarity demonstrate the strong and selective affinity of the charcoal column towards "dioxin-like" structures. Additional testing conducted with the spiked egg samples showed similar but more complex distributions of the toxic congeners in the Aroclor mixtures. Results obtained by either the GC/ECD or the GC/HRMS methods produced a successful determination of the targeted analytes maintaining an overall uniformity of recovery for all congeners (67 to 112%).

While the isolation of mono-ortho PCB's, planar PCB's, PCDD's and PCDF's indicate the existence of very narrow and well defined elution windows from the charcoal column, higher ortho substituted congeners showed an increasing overlap of the elution patterns that may complicate the separation.

The use of 13C-labeled standards combined with the resolving power of the HRMS allows the determination of the mono-ortho and non-ortho PCB's with low detection limits and greater sensitivity. However, the lack of labeled standards for the di-ortho PCB congeners makes the determination by isotope dilution extremely difficult and prone to interferences. For these reasons, the combined use of the HRGC/ECD and the HRGC/HRMS methods is recommended to achieve the desired selectivity with acceptable detection limits.

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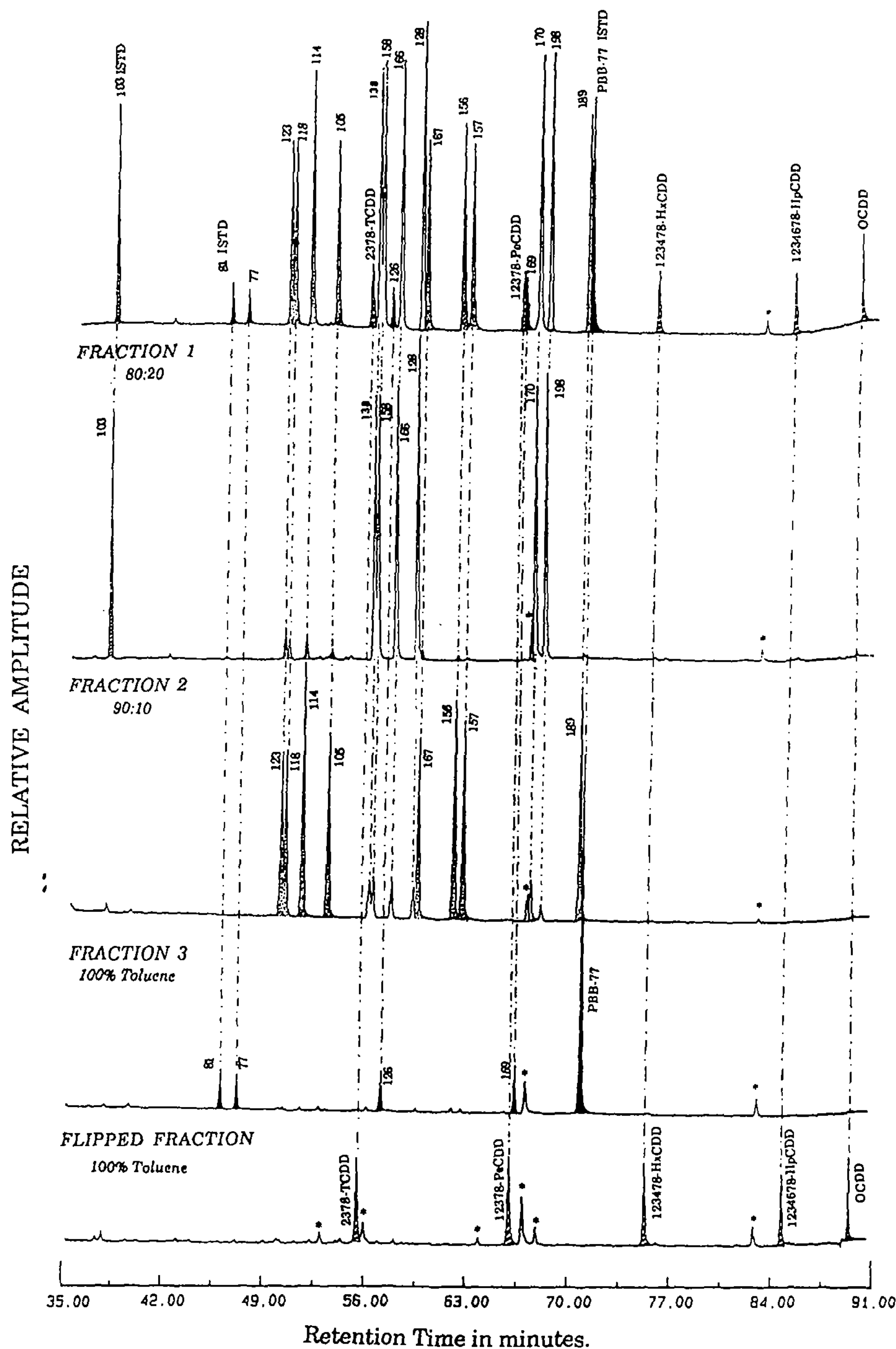

Figure 1.- Selective separation of toxic polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans using an AX-21 Super Activated Carbon/Silica gel chromatographic column. (* : interfering compound)