HPLC method with two coupled columns for the separation of PCDD/Fs, non-ortho-PCBs, mono-ortho-PCBs, di-tetra ortho-PCBs and PACs.

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Introduction

Clean-up procedures of complex samples for the subsequent analysis of compounds like PCDD/Fs, PCBs, and PACs are in most cases both time-consuming and associated with problems concerning recoveries of the compounds. These problems are in many cases due to the many steps involved in the clean-up procedures necessary for the removal of the unwanted contaminants in complex environmental matrices. One way to solve these problems is to use as closed clean-up systems as possible with as little manipulation as possible from the operator.

In this abstract we present the results of a new high performance liquid chromatography (HPLC) separation method where an aminopropyl-column (NH_2-column) is used in combination with a porous graphitic carbon (PGC) column in an automated mode. The group separation properties (according to the number of aromatic rings) of the amino column when operated in a straight phase mode, has been shown in previous studies[1,2]. Further, the high selectivity of activated carbon for the separation of planar aromatic compounds has been widely utilized as a clean-up method prior to the analysis of PCDD/Fs[3], and as a packing material for HPLC use, a PGC material has been developed[4].

Experimentals

Extraction: The different environmental sample matrices to be subjected to the HPLC separation method were; different fish samples of herring and cod from the Baltic Sea, sediment samples also from the Baltic, and an electrostatic filter precipitate from a municipal waste combustion incinerator. The first two kind of samples were chosen to reflect two problematic extracts, lipid and sulphur containing samples, respectively. The electrostatic filter sample was chosen since it is known to contain all relevant congeners of PCDD/Fs.

All samples were extracted with toluene in a Soxhlet apparatus for 24 h. For the collection of water a Dean-Stark-trap was placed on top of the Soxhlet apparatus[5]. Before extraction of the fish and sediment samples eight ^{13}C-labeled PCDD/Fs standards (2,3,7,8-TCDF, 2,3,7,8-TCDD, 2,3,4,7,8-PnCDF, 1,2,3,7,8-PnCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD and OCDD) were added at levels of 200 pg of each isomer, together

with eight ^{13}C-labeled PCB standards (3,3',4,4'-, 2,2',5,5'-TCB, 3,3',4,4',5-, 2,2',4,5,5'-PnCB, 3,3',4,4',5,5'-, 2,2',4,4',5,5'-, 2,2',3,4,4',5-HxCB, 2,2',3,4,4',5-HpCB) at levels of 2 ng/isomer. The amount of standards added was adjusted to correspond to the levels present in the samples. To the electrostatic filter precipitate sample the ^{13}C-labeled PCDD/F standards and an Arochlor 1254 mixture were added. This was made in order to obtain a mixture with as many PCDD/F and PCB isomers present as possible in the same sample.

Pre-cleaning: Before the extracts can be injected on the HPLC system a pre-cleaning step is necessary in order to remove unwanted substances that can deteriorate the separating properties of the coupled columns. In this study the pre-cleaning step consisted of elution through an open silica gel column with n-hexane as solvent whereby polar compounds as well as lipid material are retained on the column[6]. The latter is obtained if the weight of the lipid material correspond to approximately 10% of the silica packing material. The silica used was deactivated with 10% water (by weight) after first activating the gel at 500 °C for 24 h.

The HPLC System: The HPLC system is composed of a Hitachi L-6200 " Intelligent" pump equipped with a gradient unit and a Hitachi L-4200 UV-VIS detector and an additional Hitachi L-6000 pump. The operation of this pump is controlled by the L-6200 pump computer. The injector is a Rheodyne Model 7125 with a 150 µl loop. The column switching system is built of one Rheodyne Model 7067 dual six port valve, one 7000P valve, one 7030P valve and one 7060P single inlet six port outlet valve. The valves are switched with air at a pressure of 110 psi, regulated by four Rheodyne Model 7163-030 solenoid valve units. These units are activated by the timer controlled contacts, programmed from the Hitachi L-6200 pump. The system also consists of an additional Rheodyne Model 5301 3-way slider valve and a Model 5300 pneumatic actuator for automatic operation. The columns used are one Millipore 250 mm x 10 mm 5µ amino column and a Shandon 100 mm x 4,7 mm Hypercarb column. All the connections between columns and switches were of stainless steel tubing except for the wash outputs where PTFE tubing was used. The solvents used (all Merck) were n-hexane, which was used as delivered, dichloromethane and toluene which were both of p.a. quality and re-distilled in glass prior to use.

Operation of the HPLC System: When the sample is injected into the HPLC system the flow is at first introduced into the amino column with n-hexane as mobile phase. The amino column, when used in a straight phase mode, separates the components according to the number of aromatic rings. The first fraction eluting is the *aliphatic/monoaromatic fraction* containing e.g. chlorinated benzenes and halogenated aliphatics. This fraction elutes with the two columns isolated from each other, directly to the sample vial. The second fraction obtained from the amino column is the diaromatic fraction containing e.g. PCDD/Fs and PCBs. This fraction, which elutes in approximately 30 ml of n-hexane, is switched onto the carbon column. When the diaromatic fraction has eluted the two columns are again isolated from each other.

After elution of the diaromatic compounds the isolated amino column is back-flushed and the *fraction containing the polycyclic aromatic compounds* (PACs), which were still retained in the amino column, elutes as one peak corresponding to approximately 10 ml of n-hexane. During the forward elution, the PAC compounds were separated within the column, and

during the back elution they are again re-concentrated. The PAC fraction is in most cases clean enough for injection on gas chromatograph/mass spectrometer (GC/MS).

The diaromatic fraction containing for example PCDD/Fs, PCBs, PCNs (polychlorinated naphthalenes) and a big variety of other compounds, is further separated on the isolated activated carbon column. First, the *PCBs with two or more chlorines in ortho position* elutes in n-hexane. This fraction is not retained to any great extent on either of the two columns and therefore the elution from the carbon column starts already when the diaromatic fraction is switched over onto the carbon column and continues after isolation of the two columns.

After elution of the first PCB fraction, the *PCBs with one chlorine atom in ortho position (mono-ortho PCBs)* elutes with 15 ml of n-hexane/dichloromethane (DCM) (1:1) (Solvent A) at a flow rate of 2 ml/min. After this second PCB fraction the carbon column is further washed with Solvent A and DCM/methanol (1:1) (Solvent B) in a gradient ranging from 100 to 80% of Solvent A. The total washing volume is 20 ml.

When the mono-ortho PCBs have eluted the solvent is changed to toluene and the flow direction is reversed. The *PCDD/Fs and the non-ortho PCBs (co-planar)* elute in 60 ml of toluene. During the back-flushing of the carbon column the temperature is held constant at 40°C. We have found that the elevated temperature increases the recoveries of OCDD and OCDF in the first 40 ml of toluene from approximately 30 % to nearly a 100 %.

The PCDD/F and non-ortho PCB fraction is in most cases ready for injection on GC/MS. After elution of the last fraction the carbon column is washed in the reversed direction with 40 ml of toluene, 20 ml of Solvent A, 30 ml of methanol/DCM (1:1) and finally, once again with 20 ml of Solvent A. This procedure is followed by elution in the forward direction with 30 ml of Solvent A. At this point the two columns are again connected and both columns are equilibrated together with 30 ml of n-hexane.

GC/MS analysis: Evaluation of the HPLC separation method was performed on GC/MS. For PACs and PCBs this was done on a Hewlett Packard (HP) 5890 Series II GC (with a 25 m x 0,22 mm SE-54 fused silica capillary column) coupled to a HP 5971A mass selective detector. As recovery estimation standards for the PACs and the PCBs were used β,β'-binaphthyl and ^{13}C-2,2',4,4',5,5'-HxCB (IUPAC 153) respectively.

For the PCDD/Fs the analyses were performed on a VG 70E mass spectrometer and a HP 5790 GC with a 30 m x 0.25 mm SP-2331 capillary column (Supelco). The MS-system was tuned to 10000 in resolution and was used in MID-mode. With the time divided into four groups. Two ions were monitored for each congener group plus one ion for the ^{13}C-labeled standards. As recovery estimation standards for the PCDD/Fs were used ^{13}C-labeled 1,2,3,7,8-PnCDF.

The evaluation was made by analyzing both standard compound mixtures and the different environmental samples run through the HPLC system. The standard mixtures used were:

one mixture of 28 polycyclic aromatic compounds (ranging from phenanthrene to coronene, i.e. three- to six ringed compounds),

one mixture of 20 PCB compounds (containing the ^{13}C-labeled PCBs listed in the section headed 'Extraction' and their corresponding native isomers, plus 2,4,4'-TriCB, 2,3',4,4'-TCB, 2,3,3',4,4'-PnCB, and 2,3',4,4',5-PnCB).

one mixture of 25 PCDD/F compounds (containing the ^{13}C-labeled PCDD/Fs listed in the section headed 'Extraction' and their corresponding native isomers, plus 1,2,3,4-TCDD, 1,2,3,8,9-PnCDF, 1,2,3,7,8-PnCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8,-HxCDD, 1,2,3,4,6,7,8-HpCDF and OCDF).

As environmental sample matrices were used; an electrostatic filter precipitate from a municipal waste combustion incinerator to which a Arochlor 1254 had been added, different fish samples of herring and cod from the Baltic Sea and sediment samples from the Baltic Sea. The standard mixtures were analyzed in triplicates and the fish and sediment samples were analyzed in duplicates.

Results and Discussion

The recoveries of the compounds of the different fractions were between 50 and 110% for both the samples and the standard mixtures. The HPLC separation method presented here proved to be very useful for all types of sample matrices analyzed. The sulphur content in bottom sediment samples often causes problem in the GC/MS analysis of the PCBs and PACs. When using the HPLC method, however, the sulphur is no longer a problem since it elutes together with the aliphatic/monoaromatic fraction.

Finally, the total separation between the different groups of PCBs eluting from the HPLC, i.e. non-ortho PCBs, mono-ortho PCBs and the di-tetra-ortho PCBs, facilitates the possibility of later separate analysis of these compounds.

References

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