An Automated Two-Dimensional HPLC Separation Method for the Analysis of PCDD/Fs, PCBs and PAHs

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INTRODUCTION

In this paper an automated separation method is described for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polycyclic aromatic compounds (PAHs) and related compounds in complex environmental matrices. Clean-up procedures for subsequent analysis of PCDD/Fs, PCBs and PAHs are in most cases very time-consuming, why there is a great demand for rapid and effective fractionation methods. This high performance liquid chromatograhy (HPLC) method utilises a coupled column system consisting of a nitrophenylpropylsilica (nitro) column (Nucleosil, 5 µm, 250x4.6 mm) and a 2-(1-pyrenyl)ethyldimethylsilylated silica column (PYE, Cosmosil, 5 µm, 150x4.6 mm). The columns were used in a straight phase mode and combined the group separating properties of the nitro column with the selectivity for planar aromatic compounds of the PYE column. This HPLC system provides five well-defined fractions, ready for injection on GC/MS. The fractions were: *aliphatics/monocyclic aromatic compounds, mono-tetra ortho PCBs, non-ortho PCBs, PCDD/Fs and PAHs.* The HPLC system was evaluated by using both environmentally collected samples and standard solution mixtures containing a wide range of PCBs, PCDD/Fs and PAHs.

EXPERIMENTALS

Extraction and clean-up

The environmental samples were all similarly pre-treated. All samples were extracted with toluene for 24 h using a Soxhlet apparatus equipped with a Dean-Stark trap for removal of the water. Prior to extraction eight ^{13}C-labelled PCB standards, 2,2´,5,5´-TCB (IUPAC #52), 3,3´4,4´-TCB (IUPAC #77), 2,2´,4,5,5´-PnCB (IUPAC #101), 2,3',4,4',5-PCB (IUPAC #118), 3,3´,4,4´,5-PnCB (IUPAC #126), 2,2´,3,4,4´,5-HxCB (IUPAC #138), 3,3´,4,4´,5,5´-HxCB (IUPAC #169), 2,2´,3,3´,4,5,5´-HpCB (IUPAC #180) were added to the samples, together with eight ^{13}C-labelled PCDD/F 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). To the samples in which PAHs were analysed, four PAH-standards (2-methylanthracene, picene, d_{12}-perylene, dibenzo(a,i)pyrene) were added before Soxhlet extraction.

Prior to the HPLC fractionation, the toluene extract was volume reduced and eluted through a column with 10% deactivated silica (SiO_2) gel. The SiO_2 column was eluted with n-hexane and retains polar compounds as well as lipids. This pre-cleaning step is necessary in order to remove unwanted substances that can deteriorate the separating properties of the columns.

The HPLC system

In the present study a nitro column was used in combination with a PYE column. The system was used in a straight phase mode with n-hexane and dichloromethane as mobile phases. When operated in a straight phase mode, the nitro column shows the same retention properties as the aminopropylsilica column (according to the number of aromatic rings)[1,2] but is somewhat more stable and more reproducible, regarding retention times, than the aminopropylsilica column. The selectivity of the PYE column for planar aromatic compounds has been shown previously by Haglund et al.[3].

The HPLC fractionation system consisted (apart from the two columns) of two pumps, a Hitachi L-6200 intelligent pump equipped with a gradient unit and an additional Hitachi L-6000 pump. The operation of the additional pump was controlled by the L-6200 pump computer. Further, the system was composed of Hitachi L-4200 UV-VIS detector operating at 254 nm and a Rheodyne 7125 valve injector with a 180 µl loop. The column switching systems was built of Rheodyne Type 70 pneumatically-operated switching valves. This column switching system has been described earlier by Zebühr et al.[4].

The samples were injected into the HPLC system on the nitro column with n-hexane as mobile phase. Here, *three fractions* were obtained, of which one was further separated on the PYE column. *The first fraction* (containing aliphatics/monocyclic aromatics) eluted, with the two columns isolated from one another, directly from the nitro column to the sample vial. *The second fraction* eluting from the nitro column, contains aromatic compounds with two aromatic rings in their chemical structure (e.g. PCBs, PCNs and PCDD/Fs), here referred to as the diaromatic fraction, was switched onto the PYE column for further separation. After the diaromatic fraction had eluted from the nitro column, the two columns were again isolated from one another. The isolated nitro column was then back-flushed to elute *the third fraction,* the PAHs, still retained in the nitro column.
The diaromatic fraction was further separated on the PYE column and provided *three fractions. The first fraction*, eluting with n-hexane, contained the mono-tetra ortho PCBs. *Secondly*, the ortho PCBs were eluted with n-hexane. When all the PCBs have eluted, the PYE column was back-flushed with dichloromethane in order to elute *the last fraction*, the PCDD/Fs.

GC/MS analysis
The HPLC separation method was evaluated by GC/MS. For mono-tetra ortho PCBs and PAHs the analyses were performed on a Hewlett-Packard (HP) 5890 Series II GC (with a 25m x 0.22m SE-54 fused silica capillary column) coupled to a HP 5971A mass selective detector. Prior to analyses of PCBs, 2,2´,4,4´,5,5´-HxCB (IUPAC #153) was added for recovery estimations. For the analysis of non-ortho PCBs and PCDD/Fs a VG 70E mass spectrometer connected to a HP 5790 GC with a 30 m x 0.25 mm SP-2331 capillary column (Supelco) was used.
Evaluation of the HPLC method was performed by analysing standards as well as environmentally collected samples run through the HPLC system. Three standard mixtures were analysed:
one PCB mixture with 20 compounds, containing the ^{13}C-labelled PCB standards listed above (see "Extraction and clean-up") together with their corresponding native isomers, plus 2,4,4´-PCB (IUPAC #70) and 2,3,3',4,4'-PCB (IUPAC #105).
one PCDD/F mixture with 25 compounds, containing the ^{13}C-labelled PCDD/F standards listed above (see "Extraction and clean-up") together with 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.
one PAH mixture with 26 compounds, ranging from phenanthrene (three ringed compounds) to coronene (six ringed compounds).
Different abiotic (e.g. air samples, sediment samples) and biotic (e.g. samples of fish and harbour porpoise) sample matrices were analysed to evaluate the HPLC system.

Results and discussion

This two-dimensional HPLC separation method was proved to be a rapid and effective fractionation method for the analysis of PCDD/Fs, PCBs and PAHs. Five fractions (aliphatic/monocyclic aromatic compounds, mono-tetra ortho PCBs, non-ortho PCBs, PCDD/Fs and PAHs) were provided from the HPLC system, ready for injection on GC/MS. The separation was completed in 40 min and required less than 70 ml of solvents.

The method has taken advantage of earlier experiences obtained from an automated system, where an aminopropylsilica column was combined with an activated carbon column[4]. The advantages of the new system are: better long term stability for the nitro column compared to the amino column, and better chromatographic properties of the PYE column compared to the carbon column.

References

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