An Automated Cleanup Procedure with High Performance Liquid Chromatography for the Determination of PCDD/F in Environmental Samples

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Abstract

A semi-automatized clean-up procedure was developed for environmental matrices such as sewage sludge and foodstuffs using a combination of a Nucleosil- and a Cosmosil-column. The coupling of the two columns has the advantage that "backflush" elution can be used to obtain four well-defined fractions of groups of compounds. Thus, mono-tetra ortho polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), non-ortho substituted PCB, and PCDD/F can be separated. In addition, by working isocratically, our system does not need an additional HPLC-gradient-pump. Comparison with conventional discontinuously operating sample preparation techniques revealed that this new system gave excellent agreement in terms of analytical results and reproducibility. The methods was validated against standards and different environmental samples.
During the experimental period more than 500 standards and environmental samples were injected into the HPLC-clean-up system. No decrease in the sensitivity or specificity was observed.

Introduction

Analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) is expensive due to the need time-consuming procedures and of large volume of analytical grade solvents. To simplify clean-up and fractionation of PCDD/F, much research was done to develop automatic on-line cleanup procedures for different matrices. With a high performance liquid chromatography (HPLC) using a coupled two-column system it was possible to automate the sample clean-up to analyze for PCDD/F, PAH, and PCB simultaneously. Biological materials like fish [1] were cleaned-up by a combination of an alumina column with a carbon-silica column, while the clean-up of emission or sediment samples [2] was preferentially done with a nitrophenyl-propylsilica-column NO_2 (Nucleosil) followed by a 2-(1-pyrenyl) ethyldimethylsilyl - silica-column PYE (Cosmosil).

Based on a existing system previously described in the literature [2], we optimized the system in order simplify the method and to replace toxic solvents by less toxic ones.

Experimental

Sample Preparation

Before extraction, all samples were spiked with $^{13}C_{12}$-labelled PCDD/F standards (CIL:Chemical Isotope Laboratories, Woburn, MA, USA): 2,3,7,8-TCDD/F, 1,2,3,7,8-PeCDD/F, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDD/F, 1,2,3,6,7,8-HxCDD/F, 1,2,3,7,8,9-HxCDD, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDD/F, OCDD/F.

Individual samples were conventionally extracted with toluene for 24 h in a Soxhlet extractor. The extract was concentrated by evaporation. For further removal of interfering materials, extracts from food or highly contaminated emission samples were extracted with 95-97% sulphuric acid and washed with pure water. Sewage sludge was pre-cleaned by liquid column chromatography with 3% deactivated silica (63-200 mesh) as the stationary and cyclohexane as the mobile phase (see Figure 1). This pre-cleanup-procedure was necessary to separate different polar components. The so prepared sample was volume reduced to nearly 200 µl and then injected into the high performance liquid chromatography system.

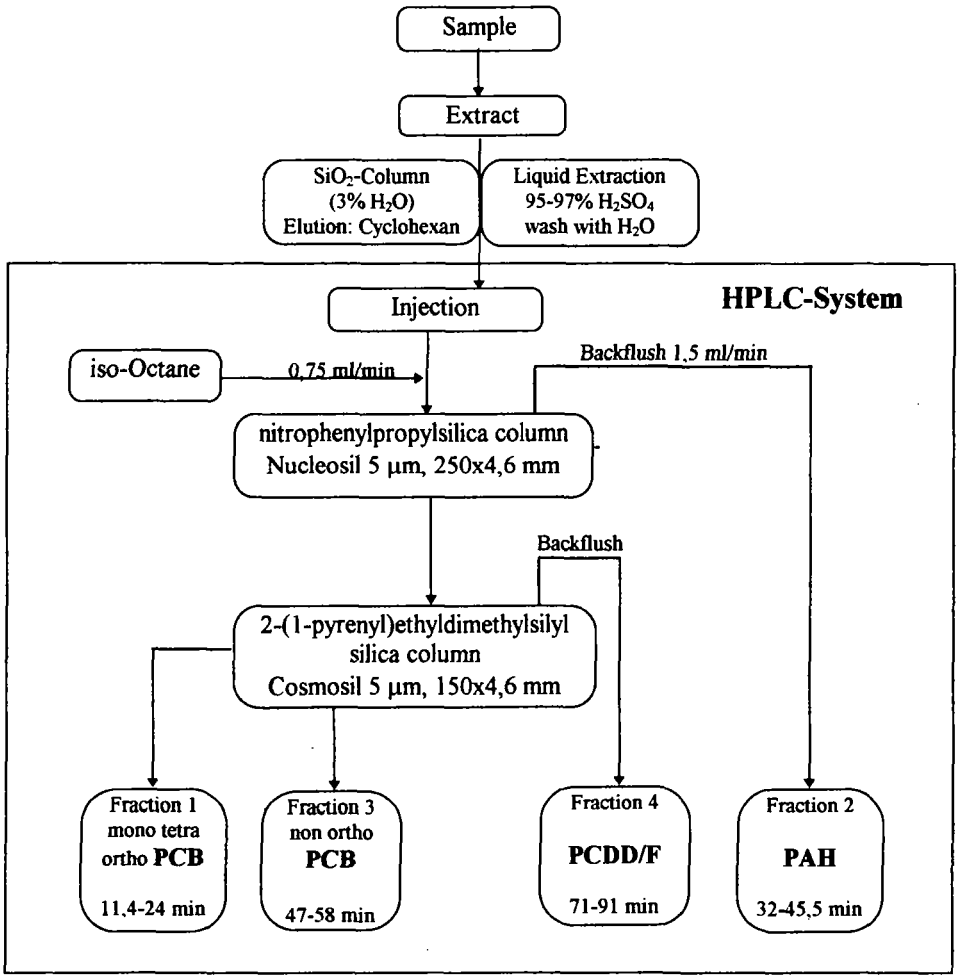


Figure 1: Schematic flow-chart of the automated cleanup-process

Clean-up with a HPLC-system

An HPLC-System from Beckmann with a simple isocratic pump (Modell 166) and an UV/VIS-detector (Modell 156) was used. Detection wsa performed at a wavelength of 254 nm. The fraction-collector (Pharmacia FRAC-100) and the pneumatic valves (Rheodyne and Dionex, V1-V2 see Figure 1) were controlled by a self constructed PC-controlled interface and a NEC P5300

computer. The clean-up program, the chromatogram and the fraction-cut were recorded by a plotter (Rikadenki). The correct switching of the pneumatic valves was by a personal computer controlled. Figure 2 shows a schematic drawing of the cleanup system. The fractions were separated by a Nucleosil 5 µm nitrophenyl-propylsilica-column, NO_2, (ID 4,6 x 250 mm, Machery- Nagel, Düren, Germany) followed by a Cosmosil 5 µm 2-(1-pyrenyl)ethyldimethylsilyl-silica-column ,PYE, (ID 4,6 x 150 mm, Nacalai Tesqne, Kyoto, Japan).
Different solvents were tested to optimize the elution parameters. Optimum results were obtained using the binary system 2,2,4-trimethylpentane (isooctane) / ethylacetate which is less toxic than the traditional solvents n-hexane / dichlormethane [2].

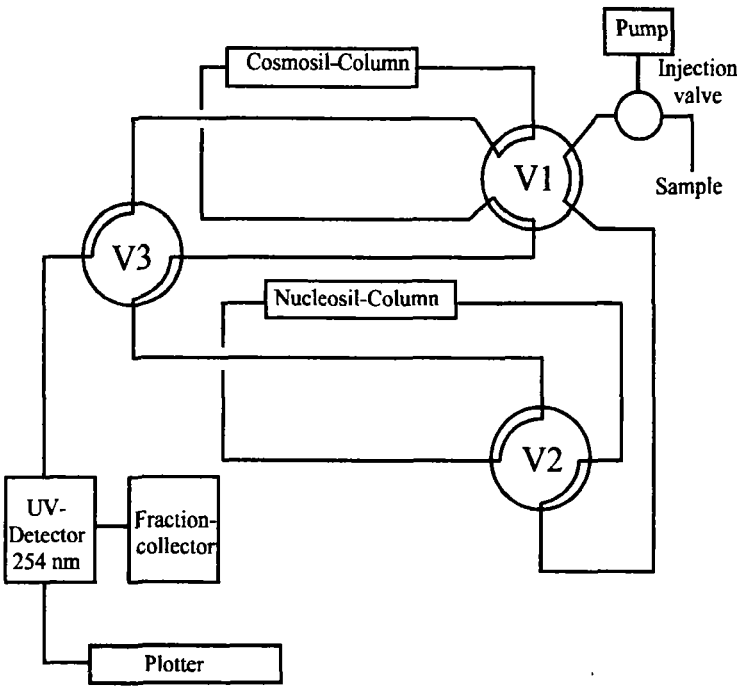


Figure 2: Scheme of the clean-up system

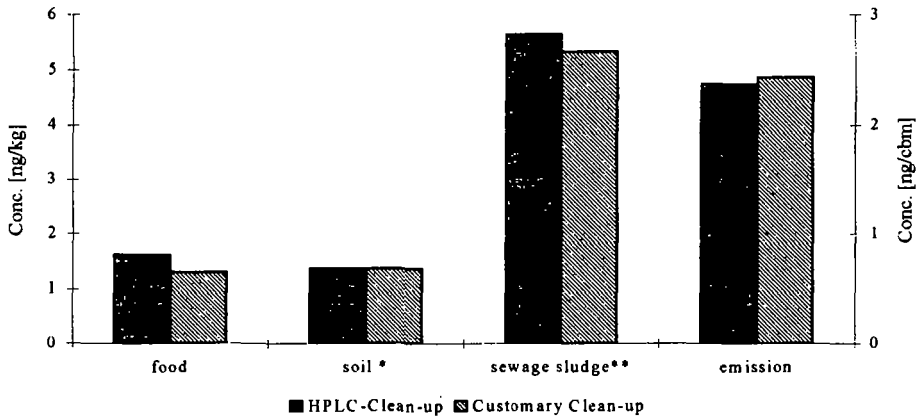
After injection, the sample was at first eluted on the Nucleosil-column, using isooctane as mobile phase. Two fractions were obtained, the first containing the ortho- and non-ortho-substituted PCB and PCDD/F and the second fraction containing the PAH (fraction 2).
To accelerate the process on the Nucleosil-column, the under this conditions little mobile second fraction was back-flushed. The first fraction was further separated on another Cosmosil-column to obtain three fractions: 1. mono-tetrachlorinated ortho-substituted PCB (fraction 1); 2. Non-ortho PCB (fraction 3); 3. PCDD/F (fraction 4). Changing the solvents from isooctane to a mixture of ethylacetate / isooctane (50/50 vol/vol%), the PCDD/F were eluted by back-flush from the now disconnected Cosmosil-column (see Figure1). Finally, reconditioning of the column was performed with isooctane (100%). After 120 min the whole clean-up process was finished.

GC/MS-Analysis

PCDD/F were separated and identified by HRGC/HRMS (Fisons HRGC/HRMS-VG Autospec) using a DB-5MS, ID 0.25 mm x 60 m column (J&W Scientific Products GmbH) or a RTX 2330, ID 0.25 mm x 60 m capillary column, according to VDI Method 3499.

Results and Discussion

In this project, different matrices, e.g. food-samples like corn and wheat, sewage sludge, soil, and emission samples were analyzed by a newly developed automatic HPLC-clean-up system and compared with the customary liquid-column clean-up. Within these tests, matrices of low and high PCDD/F contamination were used: e.g. food samples had 1,6 ng/kg PCDD/F and sewage sludge had 5637 ng/kg. The results from the different matrices obtained by our new system and in comparison to customarily processed samples are shown in Figure 3. The general agreement between both methods was very good.



* conc. x100, **conc. x1000

Figure 3: Comparison of HPLC- and customary Clean-up for different sample-matrices.

The best agreement wase obtained for the emission (flue gas of waste incineration plant) and the soil samples where the maximum difference was about 2%. For the sewage sludge sample we found about 7% higher concentrations of total PCDD/F with the HPLC method than with the traditional clean-up. For all samples and using the HPLC method, the recovery of the ^{13}C-labelled standards ranged 75-95%.

To conclude, the newly developed automatic HPLC-cleanup system can be applied for a wide range of PCDD/F concentrations. Comparison with traditional clean-up systems revealed less than 10% difference which is inside the range of analytical precision. The new system is applicable to a large variety of sample matrices and can be used without any modifications.

Between the clean-up of the different samples standards were injected in the HPLC-system to analyse the blank value of the clean-up system. An amount for a PCDD/F-concentration of 0.016 ng/kg allows the clean-up of samples with very low PCDD/F contamination e.g. food samples.
The system exhibited excellent reproducibility. In five consecutive runs of sub-samples from one emission sample using either the HPLC method or the traditional method, we found a standard deviation of 1.6 % for the HPCL-clean-up system and 1.9 % for the customary clean-up.

The analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans, especially the clean-up procedure, is one of the most expensive and time consuming processes. So the minimization of time and cost is of great interest. Here a clean-up system is presented that in comparison with the customary cleanup procedure (according to VDI 3499) [3] needs less than half the time. Inclusive pre-cleaning a sample preparation with this system needs about 4-6 hours, instead of 2 hole days with the customary cleanup to be ready for HRGC/HRMS quantification. From the standpoint of economy and ecology we will see that the automatic cleanup system needs less solvents and adsorbents and less personal attention. The main advantage of this method is not only a reduction of the solvent's volume and of time in relation to conventional clean-up processes but also the high reproducibility of the method. It could be shown that this new system can replace the customary liqid-column chromatography methods for clean-up procedures in routine analytical laboratories.

References

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[3] VDI-Richtlinien, Messen von Emissionen, Messen von polychlorierten Dibenzo-(p)-dioxinen (PCDD) und Dibenzofuranen (PCDF), VDI 3499 Blatt 2. Verein Deutscher Ingenieure, Düsseldorf, Germany **1993**