

New High Capacity Disposable Silica Columns (HCDS) instead of GPC for Automated Clean-up of Biological Fatty Matrices in PCDD/Fs and cPCBs Analysis.

Jean-François Focant, Gauthier Eppe and Edwin De Pauw

Mass Spectrometry Laboratory, University of Liege, B6c Sart-Tilman, B-4000 Liege, Belgium

e-mail : JF.Focant@ulg.ac.be Tel : 0032 4 366 35 31 Fax : 0032 4 366 34 13

Introduction

Due to their toxicity for humans (1), dioxins [polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F's)] and dioxin-like polychlorinated biphenyls [non-ortho (coplanar or cPCBs) and mono-ortho PCBs] have to be monitored in biological matrices. The acute lipophilicity of these compounds combined to relatively low amounts (ppt or less) present as contaminants makes their analysis very complex. A multi-step procedure consisting in sample extraction, adsorption chromatography columns clean-up and, finally, analysis using Gas Chromatography coupled with High Resolution Mass Spectrometry (GC/HRMS) is necessary in order to isolate and quantify these analytes (2,3).

The aim of the extraction step is to isolate the lipid fraction containing compounds of interest. Few grams of lipids are usually necessary to permit the quantification of dioxins. After gravimetric determination of the lipids content, fats have to be removed to allow analysis. Several possible routes such as acidic digestion (4), saponification (5,6), acidic silica columns (7,8) or Gel Permeation Chromatography (GPC) (9,10) are possible to carry out the lipid removal. Among them, GPC separation presents the advantage to be used repetitively without regeneration and being quite easily automated. Last years, an automated clean-up system (Fluid Management Systems, Inc., Power-Prep SystemTM) has been developed in order to increase the number of samples treated simultaneously (11,12). The load of up to 1g of lipids is generally permitted on the system. The major part of the extracted fat has to be removed before automated clean-up.

A new High Capacity Disposable Silica column (HCDS, FMS Inc.) is proposed to overcome the need of a preliminary GPC run before the automated clean-up. This results in a single automated clean-up step between extracted fat (up to 6g) and the evaporation before HRGC/HRMS injection. This study focuses on the comparison between these HCDS columns and the GPC purification.

Materials and methods

Extraction

Eggs (yolk), adipose tissues (pork and poultry), mackerel (filet) and sperm whale (blubber) were grounded under liquid nitrogen (Air Liquide, Liege, Belgium), freeze-dried and extracted using Accelerated Solvent Extractor (ASETM 200, Dionex, Sunnyvale, CA, USA). Lipids content were determined gravimetrically after extraction and aliquots of about 4-5g fat were used for each test. Dairy fat and "in house" QC (beef fat fortified with the 17 PCDD/Fs to have a content of about 8 pg TEQ/g Fat) were directly processed on HCDS or GPC.

Clean-up

GPC purification has been carried out on a Latek LC-12-3 column (Latek, Eppelheim, Germany) filled-out by 70g of S-X3 Bio-BeadsTM (Bio-Rad Laboratories, Nazareth, Belgium) using ethyl acetate/cyclohexane 1:1 as solvent.

HCDS columns (28g acidic, 16g basic, 6g neutral) were directly connected to the first column of the Power-Prep SystemTM. Samples were diluted in 50 ml of hexane.

Automated multi-columns clean-up has been performed on the Power-Prep SystemTM. All solvents were for pesticides analysis (ACROS, Geel, Belgium).

Analysis

GC/HRMS analysis (isotopic dilution method) were performed using a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany) and a Hewlett-Packard (USA) 6890 Series gas chromatograph equipped with a DB-5MS (30m x 0.25mm x 0.25µm) capillary column (J&W Scientific, Folsom, CA, USA). Procedural blanks (both instrumental and method) and quality control samples were included in the analysis to ensure that the analytical system is maintained under control. TEQs for all congeners were calculated using 2,3,7,8-TCDD TEFs reported by the WHO (1998) (1).

Results and Discussion

Our quality control chart has not shown any significant change for QC samples purified on HCDS regarding GPC samples. Some representative values of the QC chart (95% control limits) illustrated in Figure 1. These only indicate a light tendency of under estimation for HCDS. Figure 2 shows that the tendency is the same for each congeners. This has also been observed for all the matrices considered and the HCDS results were always between 1% and 8% lower than GPC ones (except for sperm whale, 14%).



Fig. 1 : Quality control chart "in house QC", n ? represent GPC and s ? represent HCDS



Fig. 2 : Values for GPC and HCDS on a congener basis for the QC

All tests were carried out in triplicates. RSDs for HCDS are higher than those of GPC (Table 1) but are still good for acceptable reproducibility. Justness values are very close to 100% for both methods.



Table 1 : RSD and Justness values for GPC and HCDS for the QC

Recoveries are very similar in both cases as illustrated in Figure 3.



Fig. 3 : Recovery pattern for each congener for the QC

Many different real samples (eggs, pork, beef, poultry, mackerel, sperm whale, dairy fat, ...) have been successfully tested and some examples for 2 very different matrices (levels of contamination for other matrices are exposed elsewhere¹³) are presented in Figure 4 and 5.



Fig. 4 : HCDS and GPC results for home-produced eggs



Fig. 5 : HCDS and GPC results for sperm whale blubber (bottom)

No significant differences were observed between blank values and no increase in the GC/MS background has been observed. Risks of cross contamination are

however reduced due to the disposable character of the HCDS. A single wash step was sufficient to avoid carry-over on the Power-Prep System™, even when 4g of sperm whale fat were processed before lower contaminated samples. Reproducibility, repeatability and robustness have been evaluated and are very good.

As for the QC, percentage recoveries for all samples (excepted for dairy fat) processed through the entire clean-up procedure were very close for both techniques. In the case of dairy fat, the acidic silica treatment seems to be more suitable than size exclusion separation for removing the lipids.

An important point to consider is also the solvent consumption and the time required and the global cost for the clean-up step. Including all the parameters, the price of one run is roughly the same while the solvent consumption is reduced of about a half. The sample capacity is however increased drastically when a five lines Power-Prep System™ is used. The same operator can then process several samples in parallel and the time required for the total clean-up step is nicely reduced.

This system avoids the purchase of additional high cost automated GPC equipment which would anyway not be so fast than the HCDS system. The complete system using disposable columns don't require skilled personnel. Only small and fast training is necessary.

Conclusions

The proposed clean-up system allows a single operator to carry out up to 10 samples a day from extraction to final concentration before MS analysis. The effectiveness of the new HCDS columns coupled with the robustness of the Power-Prep System™ make this combination a powerful tool for low contaminated high fat content matrices analysis.

In addition to PCDD/Fs and cPCBs, this system is also able to isolate mono-ortho PCBs.

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