

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

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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 System™) 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 (ASE™ 200, Dionex, Sunnyvale, CA, USA). Lipids content were determined gravimetrically after extraction and aliquots of about 4-5g fat were used for each test.

ORGANOHALOGEN COMPOUNDS

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-Beads™ (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 System™. Samples were diluted in 50 ml of hexane.

Automated multi-columns clean-up has been performed on the Power-Prep System™. 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. The QC chart (95% control limits) illustrated in Figure 1 only indicates a light tendency of under estimation for HCDS. 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%).

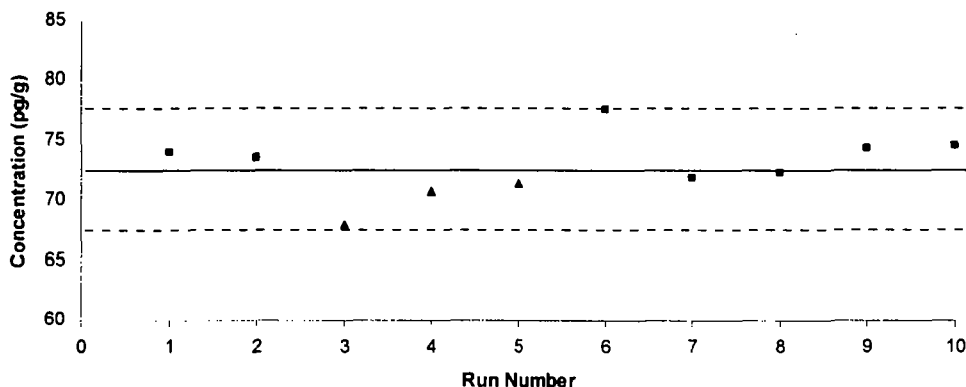


Fig. 1 : Quality control chart "in house QC", ■ represent GPC and ▲ represent HCDS

All tests were carried out in triplicates. In HCDS, RSDs of measures were always lower than those of GPC, this indicates that HCDS step is more reproducible. Figure 2 illustrates the results for 2 very different matrices (levels of contamination for other matrices are presented elsewhere¹³).

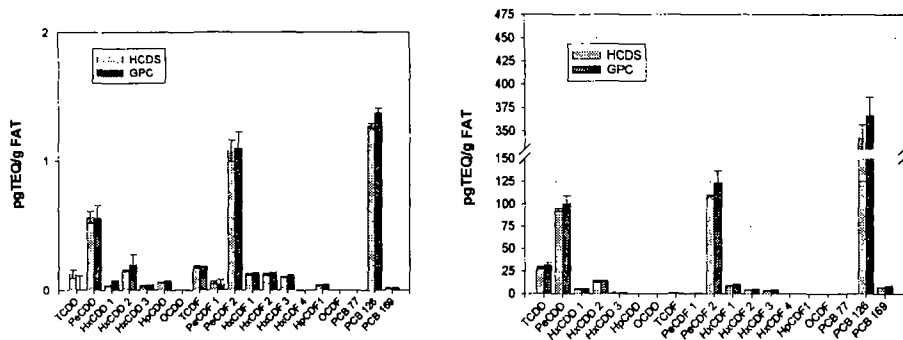


Fig. 2 : HCDS and GPC results for home-produced eggs (left) and sperm whale blubber (right)

No significant differences were observed between blank values. 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.

Percentage recoveries for all samples (excepted for dairy fat) processed through the entire clean-up procedure were very close for both techniques (Table 1). In the case of dairy fat, the acidic silica

treatment seems to be more suitable than size exclusion separation for removing the lipids. In most cases, the values of the relative standard deviations for recoveries are lower for GPC runs. This can be due to some small homogeneity problems in the packing of the disposable columns.

Table 1 : Comparisons of recoveries for QC and Dairy fat samples

	QC		Dairy fat	
	HCDS % (RSD)	GPC % (RSD)	HCDS % (RSD)	GPC % (RSD)
2,3,7,8-TCDD	93 (26)	67 (13)	110 (6)	78 (10)
1,2,3,7,8-PeCDD	71 (15)	75 (7)	84 (17)	60 (17)
1,2,3,4,7,8-HxCDD	67 (18)	68 (12)	73 (16)	50 (20)
1,2,3,6,7,8-HxCDD	66 (10)	63 (10)	68 (21)	41 (14)
1,2,3,7,8,9-HxCDD	66 (13)	70 (13)	76 (19)	51 (14)
1,2,3,4,6,7,8-HpCDD	87 (14)	77 (3)	88 (18)	75 (6)
OCDD	65 (46)	54 (43)	82 (16)	64 (11)
2,3,7,8-TCDF	87 (11)	84 (6)	115 (17)	80 (3)
1,2,3,7,8-PeCDF	71 (19)	78 (8)	95 (19)	71 (13)
2,3,4,7,8-PeCDF	56 (4)	61 (3)	92 (9)	61 (5)
1,2,3,4,7,8-HxCDF	71 (20)	76 (13)	88 (17)	62 (5)
1,2,3,6,7,8-HxCDF	73 (12)	75 (12)	85 (17)	54 (9)
1,2,3,7,8,9-HxCDF	74 (17)	72 (14)	89 (18)	57 (8)
2,3,4,6,7,8-HxCDF	72 (17)	74 (17)	90 (19)	58 (5)
1,2,3,4,6,7,8-HpCDF	81 (13)	79 (5)	100 (16)	88 (18)
OCDF	62 (48)	62 (26)	97 (17)	71 (17)
3,3',4,4'-TCB	77 (5)	89 (6)	95 (14)	66 (3)
3,3',4,4',5-PeCB	71 (33)	67 (27)	90 (16)	53 (9)
3,3',4,4',5,5'-HxCB	66 (6)	77 (14)	73 (25)	47 (9)

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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