Cod: 2.1030

A LOW COST EFFECTIVE CLEAN-UP METHOD FOR DIOXIN ANALYSIS

K. Kedikoglou[1], D. Costopoulou[1], I. Vassiliadou[1], L. Leondiadis[1]

[1]*Mass Spectrometry and Dioxin Analysis Laboratory, NCSR "Demokritos", Athens, Greece*

Introduction

The official analytical methods of the EU for the control of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) include screening and confirmatory methods[1,2]. The accurate analysis of PCDD/Fs and PCBs requires the prior separation of the analytes from the undesirable interferences. This, in the case of food and feed samples, is achieved through a combination of lipid extraction followed by several steps of chromatographic separation using sorbents such as silica, alumina and Florisil. The final and crucial step of separation of PCDD/Fs from PCBs is usually achieved by carbon based materials, which have a strong affinity for planar aromatic compounds such as PCDD/Fs and PCBs. The strongly retained analytes are removed from the carbon columns by back-flashing techniques or by elution by large volumes of appropriate solvents. Several carbon materials have been used for this purpose: Amoco PX-21, Carbosphere, Carbopack B and C. A comparative study of the above materials, established that all of them are able to separate PCDD/Fs from PCBs adequately, though in some cases they present difficulties in separating different PCB congeners or have low recoveries in highly substituted chlorophenyls[3].
Automated systems have been developed, using a combination of column separations by silica, alumina and carbon and appropriate solvents, the most widely used being the commercially available Powerprep® system by Fluid Management Systems, Inc[4]. Nevertheless, due to the high cost of the system itself, its disposable columns and the high amounts of solvents required, it is not an affordable solution for all laboratories, therefore manual clean-up methods are still an attractive alternative. A manual clean-up method, initially developed in 1990 by Liem et al[5] and improved in 2004 by Papadopoulos et al[6], was based on the use of Carbosphere as an activated carbon material. This material offered the advantage of high fat capacity, which was important for food items with high fat content and low analyte concentrations. However, Carbosphere presented some drawbacks, the most important of which was the fact that it was expensive and had to be regenerated and reused, which leaded to the appearance of high background levels after several cycles of application. Moreover, Carbosphere is no longer commercially available. This study presents an alternative active carbon material, namely FU 4652, which can be used in the isolation of PCDD/Fs and PCBs. Similarly to Carbosphere, this material can be used in samples containing high amounts of lipids (even higher than 10 g), but its considerably lower cost allows it to be disposable, which eliminates the development of undesired background. Its evaluation showed that it is capable of separating all analyzed congeners with good recoveries. Samples analysed for the evaluation of the above active carbon materials were olive oil reference samples spiked with PCDD/Fs and non-ortho PCBs at two concentration levels.

Materials and methods

Samples

Reference samples analyzed were prepared by the addition of known amounts of PCDD/Fs (0.5 pg/g and 1 pg/g) and non-ortho PCBs (12.5 pg/g and 25 pg/g) in virgin olive oil.

Materials

All solvents used were residue analysis picograde and were purchased from Promochem (Germany). The activated carbon FU 4652 was purchased from Schunk Kohlenstofftechnik GmbH. According to the specifications provided by the producer, its specific surface area is 1400 m^2 $g^{-1}$and mean grain size is 600 μm. Alumina was Basic Activity Super 1 for dioxin analysis, MP Biochemicals GmbH. The sulfuric acid impregnated silica gel (44 % H_2SO_4-silicagel) was prepared with Silica gel 60-200 mesh (Merck), activated in an oven at 200 ^oC mixed with concentrated sulfuric acid. The internal quantification standards used were ^{13}C-labelled solutions of PCDD/Fs and PCBs in toluene and were added to each sample prior to clean up. They contained a mixture of $^{13}C_{12}$ isomers of all the 17 PCDD/Fs congeners except OCDF and 4 $^{13}C_{12}$ non-ortho PCBs. The isomers for the preparation of the $^{13}C_{12}$ internal and injection standard solutions were purchased from Wellington Laboratories (Canada).

Clean-Up

Carbon chromatography

A glass column (length 10cm, 10mm, 10mm ID) equipped with mounting ends on both sides was initially filled with glass wool, 1.8 g of activated carbon FU 4652 and another layer of glass wool. The column was connected to a glass funnel. The olive oil samples were dissolved in 50 mL of dichloromethane and brought onto the top of the carbon column. The carbon column was placed in a reflux unit and refluxed for 2 h with 30 mL of dichloromethane. This fraction including residual fat was discarded. Next the column was rinsed with 20 mL of toluene and refluxed with 30 mL of toluene for 1, 1.5 and 2 h. This fraction containing the non-ortho PCBs and was evaporated to dryness. The carbon column was then inverted in the reflux unit and the PCDD/F fraction was eluted from the column by refluxing with 40 mL of toluene for 16 h. This fraction was evaporated to dryness as well.

Alumina chromatography

Detailed procedure has been described elsewhere[6]. Further clean-up was performed by basic alumina chromatography. Final eluates were evaporated to dryness and re-dissolved in 50 μL of the appropriate ^{13}C-labelled injection standard.

Instrumental Analysis

The quantification of non-ortho PCBs and PCDD/Fs was performed by High Resolution Gas Chromatography-High Resolution Mass Spectrometry (Electron-Impact) (HRGC-HRMS) (EI), on Multiple Ion Detection (MID) mode, on a Trace 1310 gas chromatograph (ThermoFinnigan) equipped with a TriPlus RSH autosampler, coupled to a DFS mass spectrometer (ThermoFinnigan) performing at 10,000 resolving power (10% valley definition). Instrumental conditions and purity control criteria are according to EPA 1613B method (US Environmental Protection Agency, 1994) and European Commission Regulations 589/2014 and 709/2014. The quantification was carried out by the isotopic dilution method. For TEQ calculations the WHO-2005 toxic equivalency factors (TEF) were applied[7]. The limit of detection (LOD) for each congener was determined as the concentration in the extract which produced an instrumental response at two different ions to be monitored with a signal to noise ratio of 3:1 for the less sensitive signal. Values below LOD were assumed to be equal to LOD (upperbound concentrations).

Results and discussion

In this study a carbon sorbent is presented, suitable for use in a new clean-up method for dioxin analysis in matrices with high fat content, such as food samples. Our initial aim was to replace Carbosphere, which has been used for more than ten years in our laboratory for the analysis of food, environmental and biological samples, but unfortunately is not any more commercially available. Carbosphere was applied in dioxin analysis for the separation of planar and non-planar compounds initially by Liem et al[5], and the characteristic that rendered it suitable for this purpose was its high capacity for at least 10 grams of fat, which allowed the development of analytical protocols for high fat containing food products[6]. An additional purpose was to find a relatively low cost material so as to avoid sorbent recycling, which was necessary for Carbosphere due to its very high cost. The regeneration procedure required was both time and solvent consuming. Moreover, after several years of use of the Carbosphere sorbent, despite the fact that it was refluxed with methanol and toluene for 6 weeks after each use, high background signal appeared in the sample chromatograms, impeding the accurate determination of all target compounds, especially the congeners with five or more chlorine substitutions.

Other commercially available sorbents like Carbopack and Celite, proposed by the US EPA 1613 method (US Environmental Protection Agency, 1994), have the limitation of low fat capacity.

The suitability of a carbon sorbent for dioxin analysis does not depend only on its fat capacity, but also on its ability to separate planar from non-planar compounds. High selectivity and specificity is necessary in order to minimize the need for additional columns to eliminate possible interfering compounds like aromatic compounds, organochlorine pesticides, ortho-PCBs, polychlorinated diphenyl ethers etc.

The Schunk FU 4652 material is graphite based activated carbon, with a grain size of 600 μm, which was initially used by Boeing for removal of contaminants from air. According to the manufacturer, it is characterized by the spherical form of the individual particles, which are classified by size. As a result of this, the material has an ideal packing density and allows liquid substances to flow perfectly through it. Due to its high compressive strength and spherical shape, the entire surface is highly accessible. In the clean-up protocol developed in the present study, this carbon sorbent was applied as an initial clean-up step, to separate planar from non-planar compounds and to remove fat. As already mentioned, the fat

capacity of the activated carbon is an important factor for the suitability of the clean-up method for high fat containing food samples. To demonstrate this, a maximum of 10 g of fat were mounted on the FU 4652 carbon column. This fat amount was completely eluted by the amounts of solvents reported in the experimental section, which shows a relatively low affinity of FU 4652 carbon for lipids.
As shown in the following method validation data, this new methodology showed highly reproducible results and very good recoveries, for both PCDD/Fs and non-ortho PCBs. Recoveries of the labelled internal standards for PCDD/Fs and non-ortho PCBs are shown in Figures 1 and 2 respectively. Especially for non-ortho PCBs, in order to optimize recoveries, three extraction durations were tested: 1, 1.5 and 2 h. Optimum results were obtained when the carbon FU 4652 column was refluxed for 2 hours and are shown in Figure 2.

Figure 1 Percent recoveries of PCDD/Fs internal standards

Figure 2 Percent recoveries of non-ortho PCBs internal standards

The FU 4652 method was validated for its accuracy and reproducibility. Moreover, in order to assure that the new method fulfils the analytical criteria set in EU regulation 589/2014 and 709/2014, the method limit of quantification (LOQ) and specificity were also examined. Blank samples were analysed, in order to determine background levels of the extracts collected from the new sorbents. The accuracy of the new method was assessed by the addition of known amounts of PCDD/Fs and non-ortho PCBs to olive oil, at two concentration levels of 0.5 and 1 pg/g for PCDD/Fs and 12.5 and 25 pg/g for non-ortho in virgin olive oil. These concentrations correspond to TEQ concentrations of 1.69 and 3.38 pg/g for PCDD/Fs and 1.38 and 2.75 pg/g for non-ortho PCBs, applying WHO-2005 toxic equivalency factors (TEFs)[7].
To test the reproducibility of the method using FU 4652, the RSD was calculated by performing 6 replicate analyses at each concentration level. The RSD values found for PCDD/Fs and non-ortho PCBs are shown in Table 1. European Commission Regulation 589/2014 specifications require RSD < 15% and trueness values in the range of - 20% to + 20 %. As shown in Table 1, RSD and trueness values calculated in our study fulfill these specifications.

Table 1

The LOQ determined was 0.10 pg/g for tetra-, penta-, hexa- and hepta-chlorinated PCDD/Fs and non-ortho PCBs, and 0.15 pg/g for octa-chlorinated PCDD/Fs. For dioxin analysis, LOQ is equal to LOD (limit of detection).
For the method specificity assurance, relative retention times and ion abundance ratios at the m/z's defined for all congeners were checked according to U.S. EPA Method 1613 (US Environmental Protection Agency, 1994) and European Commission Regulations 589/2014 and 709/2014.
The results presented above, show that the FU 4652 activated carbon is efficient for the separation of PCDD/Fs and non-ortho PCBs. The quality criteria examined for the above method (analytes recoveries, accuracy, reproducibility, specificity and detection limits) show that the method sensitivity and specificity are adequate for the analysis of food samples.

Acknowledgements
We gratefully acknowledge Best Buy Analytical Greece and Schunk Kohlenstofftechnik GmbH for providing the FU4652 carbon sorbent.

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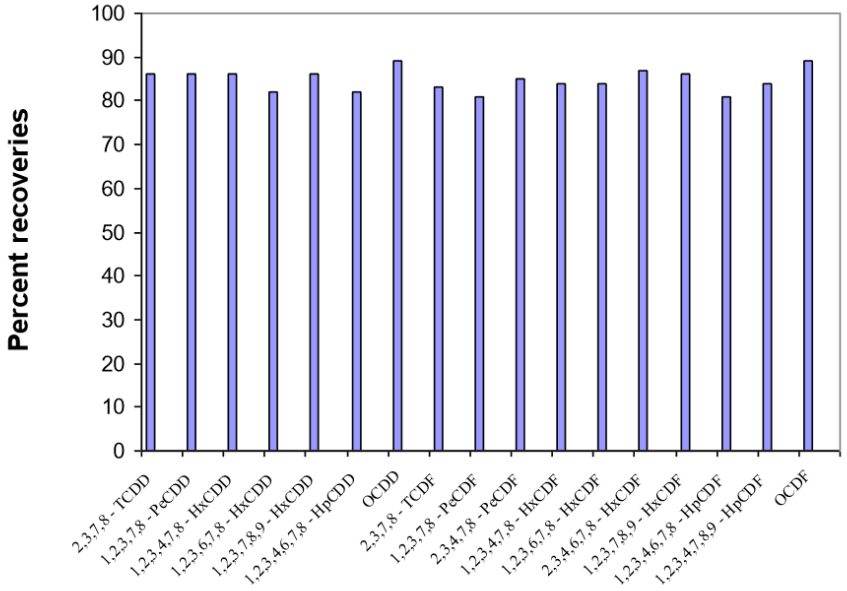

Figure 1

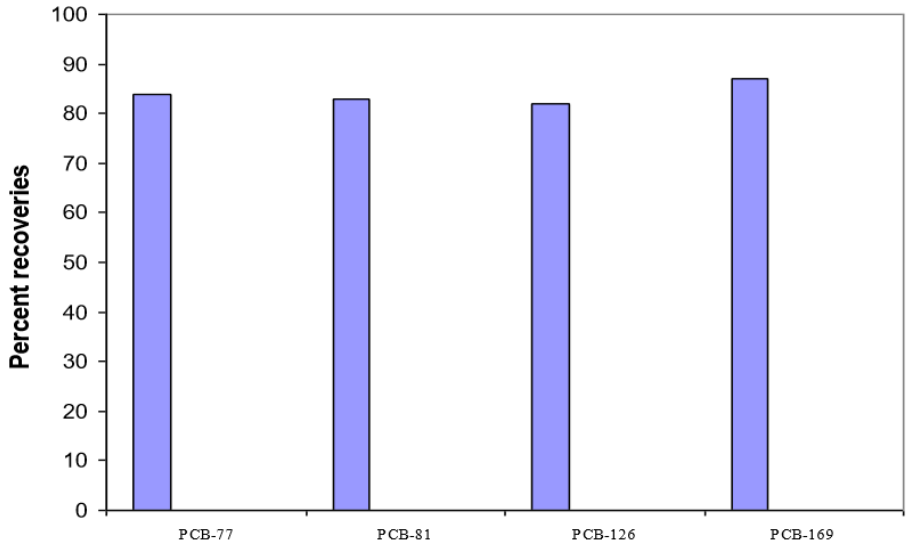

Figure 2

	PCDD/Fs		Non-ortho PCBs	
Fortification level (TEQ)	1.69	3.38	1.38	2.75
Average	1.65	3.44	1.36	2.67
SD	0.03	0.12	0.09	0.11
% RSD	1.56	3.60	6.31	4.30
% Trueness	-2.37	1.77	-1.45	-2.91

Table 1