OPTIMIZATION OF A NEW DIOXIN/PCB CLEAN-UP AND FRACTIONATION PROCEDURE FOR AN EXISTING AUTOMATED SYSTEM
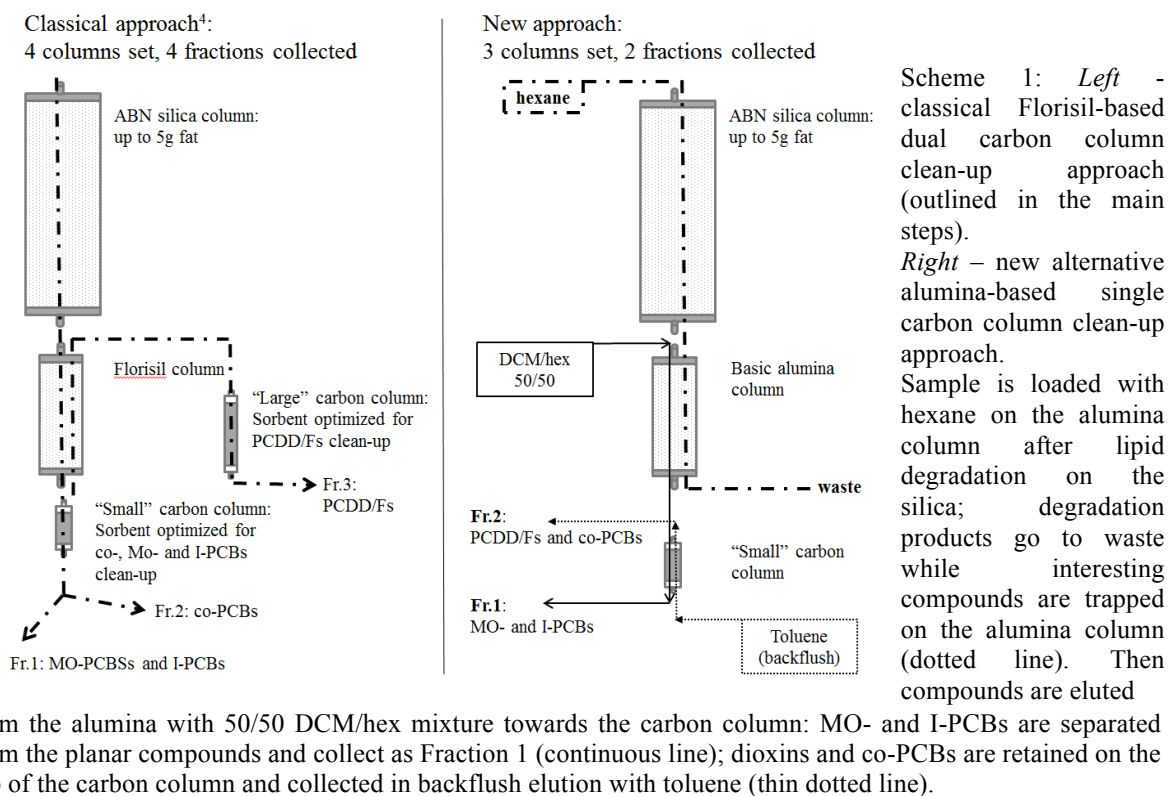
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Introduction

In the recent years PCDD/Fs and PCBs analysis has shown the need for fast and high throughput methods to identify and confirm contamination levels in feed and food samples. Very recently instrumental analysis progressed with the introduction of GC-MS/MS as confirmation method[1, 2] in addition to GC-HRMS sector instruments after showing comparable performances[3]. Sample preparation can be carried out in several ways, often involving several steps based on the use of various sorbents and solvents; different fractionation results can also be obtained, often based on the GC-MS methods to be used.

To offer more flexibility, a modified sample clean-up and fractionation procedure is proposed for the use of an existing automated system[4]. The new method indeed uses 3 columns (instead of 4) in a row: acidic silica – basic alumina – carbon column, taking advantage from the fractionation properties of the basic alumina, which is able to fractionate and to trap all the relevant compounds. The alumina is eluted with 50/50 DCM/Hex mixture through the carbon column to collect separately 2 fractions (instead of 3 of the classical approach): ppb level compounds, namely mono-ortho (MO-) and indicators (I-)PCBs, and trace level planar compounds, such as PCDD/Fs and coplanar (co-)PCBs (#77, 81, 126, 169). The described method takes 41 minutes to be performed and requires 287 mL of solvents. Fractions can be analyzed by GC-MS/MS, following the recent EU regulation. The development of the new approach is still under examination and preliminary tests have been carried out on food matrices (vegetable oil, milk, pork fat); further validation will be done with Proficiency Tests (PTs).

Scheme 1: *Left* - classical Florisil-based dual carbon column clean-up approach (outlined in the main steps).
Right – new alternative alumina-based single carbon column clean-up approach.
Sample is loaded with hexane on the alumina column after lipid degradation on the silica; degradation products go to waste while interesting compounds are trapped on the alumina column (dotted line). Then compounds are eluted from the alumina with 50/50 DCM/hex mixture towards the carbon column: MO- and I-PCBs are separated from the planar compounds and collect as Fraction 1 (continuous line); dioxins and co-PCBs are retained on the top of the carbon column and collected in backflush elution with toluene (thin dotted line).

Materials and methods

Routine chemicals and procedures for PCDD/Fs and regulated PCBs analysis have been described elsewhere, as well as instrumental analysis by means of GC-MS/MS Triple Quad instrument[3]. In addition, in this study disposable glass columns for the automated clean-up were obtained from LCTech (LCTech GmbH, Bahnweg 41, Dorfen, Germany), such as acid silica standard column (able to degrade up to 5g of fat) and two different carbon columns, with different particle size and activity, indicated as "small" column for PCBs retention and "large" column for dioxins. Basic alumina columns were manually packed with 10 g of commercial powder purchased from Sigma Aldrich (St. Louise, MO, USA). Prior to use, alumina was backed at 600°C for at least 30 hours[5] and columns were packed when alumina was still hot to prevent water adsorption and sorbent deactivation. In this preliminary study, for method development, samples mimicked blanks for fat extracts in our routine lab were injected into a 15 mL loop of a DEXTech system from LCTech by means of 10 mL borosilicate glass syringes and consisted in 2 mL of hexane spiked with all congeners of PCDD/Fs 2,3,7,8-substituted and coplanar PCBs (#77, 81, 126, 169) ^{13}C-labeled internal standard (EDF-4144, Cambridge Isotope Laboratories, CIL; Andover, MS, USA), as well as the mono-ortho (MO-)PCBs (#105, 114, 118, 123, 156, 157, 167, 189) ^{13}C-labeled standard (MBP-MXK from Wellington Laboratories; Guelph, Canada) and the indicator (I-)PCBs (#28, 52, 101, 138, 153, 180) ^{13}C-labeled standards (EC-4058 from CIL). All the experiments have been run at 7 mL/min flow rate, following the classical approach[4]. After clean-up two fractions were collected, one containing MO- and I-PCBs at ppb levels (PCB fraction, Fr. 1) and the other containing PCDD/Fs and co-PCBs at ppt levels (dioxin fraction, Fr. 2). This partition aims to separate and gather compounds having similar average levels in real samples. Recoveries were measured with recovery standards EDF-4145 syringe standard (CIL) for the dioxin fraction, and with EC-1414 solution of $^{13}C_{12}$-labeled PCB-80 (CIL) for the PCB fraction. Solvent volumes were reduced first in N_2 stream using a sensor equipped TurboVap II Workstation (Caliper Life Science, Teraflene, Belgium), and then by means of a RapidVap (Labconco, Kansas City, MO, USA) after transfer in GC vials containing nonane as keeper (respectively 90 and 4 µL for the PCB and the dioxin fraction).

Results and discussion

Development and optimization of the clean-up method have been carried out step by step measuring recovery rates after the main stages of the procedure: 1) silica and alumina columns loading; 2) alumina column elution to collect the PCB fraction (Fr 1); carbon column elution to collect the dioxin fraction (Fr. 2).

Conditioning step

Before each experiment, columns were conditioned using the smallest hexane amount after dead volume measurement, that is: 42 mL (6 min) for the silica column, 10.5 mL (1.5 min) for the alumina and 3.5 mL (0.5 min) for the carbon columns, with a total hexane consumption of 56 mL and 8 minutes for the complete conditioning of the three columns in a row. Proven the commercial columns are clean, this step can be omitted.

Silica column elution optimization

Silica column elution profile was outlined to assess the time/solvent amount required to completely empty the silica column when loading the alumina one. After injection into the loop, the sample was loaded with hexane on the silica column and aliquots were collected after 5,10,15,20 and 25 minutes. Silica elution profiles for both fractions showed that 140 mL of hexane (20 min) were needed to elute all the compounds out of the silica column towards the consequent alumina column for the fractionation.

Silica + Alumina column elution optimization

The next step was to connect in sequence the silica and the alumina columns for compound fractionation without any losses in the waste (Scheme 1, dotted line). The core of the method development was to find the suitable treatment for the alumina in order to trap all the compounds on it getting rid only of the co-extracts. After several tests, basic alumina backed for at least 30 hours at 600°C (EPA Method 1613[5]) showed desired properties. For the method development columns were manually packed with 10 g of basic alumina when the sorbent was still at ~300°C (not at 130°C as reported in the EPA 1613) to prevent moisture deactivation. Likely in the industrial production process alumina column will need to be packed in inert atmosphere and hermetically sealed to prevent water adsorption.

Manually packed alumina column, preceded by the silica column, was loaded with 140 mL of hexane going to the waste (Scheme 1, dotted line), that was collected to confirm no compound was lost. Then the alumina column was eluted with 50/50 DCM/hex mixture and fractions were collected every 2 minutes for 8 minutes to assess its elution profile and to estimate that 36 mL of 50/50 DCM/hex mixture (6 min) were necessary to elute out all the analytes from alumina column.

Silica + Alumina + Small carbon connection and overall clean-up method

The last step in the method development was to connect the 3 columns in sequence, silica – alumina and "small" carbon column (the commercial one optimized for PCBs clean-up) envisaged for the new clean-up approach (Scheme 1). The sample, 2 mL of hexane spiked with ^{13}C labelled standards was injected in the sample loop and loaded on the alumina column with 140 mL of hexane (to the waste). Then the alumina column was eluted with 36 mL of 50/50 DCM/hex mixture through the carbon column (Scheme 1, continuous line) and fractions containing MO- and I-PCBs were collected every 2 minutes for 8 minutes to assess the carbon column elution profile and hence the minimum solvent required for PCBs collection, saving solvent and time in the following evaporation step. Carbon column elution profile for the PCBs fraction was different from the corresponding alumina one, indicating the carbon did interact with the compounds, slowing down their flow; in fact 42 mL (instead of 36) of the 50/50 DCM/hex mixture were needed to collect all the MO- and I-PCBs, with recoveries ranging between 60 and 90%.

Planar compounds, loaded on the carbon column and net on its top because of geometrical interactions with the sorbent, were collected backflushing the carbon column with 28 mL (4 min) of toluene (Scheme 1, thin dotted line). Recoveries for PCDD/Fs ranged from 80 to 100% and from 70 to 90% for co-PCBs (#77, 81, 126, 169).

Time and solvent consumption.

The alumina-based method described so far was developed as an alternative to an existing automated high throughput clean-up method for dioxin analysis[4]. It seems to be promising because time and solvent consumption are very competitive (Table 1) when compared with other clean-up strategies, keeping recovery rates in the ranges accepted by EU Regulation.

	Column	End	Solvent	Time (minutes)	Volume (mL)	Hexane (mL)	DCM (mL)	Toluene (mL)
Conditioning	Si	waste	hex	6	42	42	0	0
	Al	waste	hex	1.5	10.5	10.5	0	0
	C	waste	hex	0.5	3.5	3.5	0	0
Load Al	Si - Al	waste	hex	23	161	161	0	0
Elute Al, load C, collect F1	Al - C	F1	DCM/hex	6	42	21	21	0
Backflush C, collect F2	C	F2	toluene	4	28	0	0	28
TOTAL CONSUMPTION				**41**	**287**	**238**	**21**	**28**

Table 1: Solvent and time consumption of the main stages of the alumina-based new clean-up method.

Further investigation on real samples and PTs is going on.

References:
1. COMMISSION REGULATION (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012, (2014).
2. COMMISSION REGULATION (EU) No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009 as regards the determination of the levls of dioxins and polychlorinated biphenyls (2014).
3. L'Homme, B., Scholl, G., Eppe, G. and Focant, J.F., (2015) *Journal of Chromatography A*, 1376, 149-158.
4. Bernsmann T., Albrecht M. and Fürst P., (2014) *Organohalogen Compounds*, 76, 1281-1284.
5. EPA Mthod 1613 - Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dlution HRGC/HRMS, (1994).