A FULLY AUTOMATED METHOD FOR THE DETERMINATION OF PCDD/FS, DIOXIN-LIKE PCBS, NON-DIOXIN-LIKE PCBs AND POLYBROMINATED DIPHENYL ETHERS IN FOOD, FEED AND ENVIRONMENTAL SAMPLES

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Abstract
Polychlorinated dibenzo-p-dioxin (PCDD/Fs) and polychlorinated biphenyls (PCBs) have been subject of many incidents with food and/or feed (1). Flame retardants such as poly brominated diphenyl ethers (PBDEs) have also been a rising group of interest to be analysed. One of the major problems laboratories are confronted with, especially for PCBs and PBDEs, is the contribution caused from the laboratory environment. Using comprehensive automation for the extraction and purification, background contamination can be significantly be decreased while on the other hand sample capacity can be increased. With the current available instruments up to 100 samples per week can be handled by one technician, with a turnaround time of eight hours to two days.

Introduction
Nowadays laboratories are requested not only to analyse samples for the presence of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) but to expand the scope of the analysis e.g. to dioxins, (non-)dioxin-like polychlorinated biphenyls and flame retardants such as poly brominated diphenyl ethers (PBDEs). In order to achieve low limits of quantitation, highly sensitive and specific methods are required. Prior to extraction ^{13}C labelled (internal standards) are, for identification and quantification, added to the samples. The first step of the analysis is extraction - often done by classical soxhlet. After that, obtained extracts are purified by an array of columns packed with sorbents like Silica (acidified) , Alumina, Florisil, Carbon etc. One important step during purification is separation between the different compound groups e.g. planar compounds (dioxins and NO-PCBs) and non-planar compounds (MO and NDL-PCBs) which can be carried out using e.g. an activated carbon column. Purified extracts are finally concentrated down to a small volume of typically 10-25 µl and analysed with gas-chromatography-high-resolution-mass spectrometry (HRGC-MS) or GC-MS-MS. In order to improve extraction and purification methods in terms of capacity and turnaround time while at the same time maintaining the quality, automation is a must. Since the introduction of the first automated systems for extraction and for purification additional benefits have been focussed on such as: low solvent consumption; avoiding the use of toxic solvents such as Dichloromethane; shorter sample preparation time; elimination of cross contamination and user friendliness.
A major problem in most laboratories, especially for the determination of PBDEs, is background contamination form the laboratory environment caused by the frequent use of flame retardants in furniture, equipment and fire resistant ceiling tiles. Using closed systems will contribute to lowering background levels and would therefore be preferred.

Material and method

Since 2015 a combination of an automated extraction system based on Randal principle (SER-158 Velp Scientifica, Usmate, Italy) and an automated purification system (MIURA, Matsuyama, Japan) is routinely in use for the determination of Dioxins and PCBs. (2)
To investigate whether this combination is also applicable for the determination of PBDEs the standard extraction and purification methods were tested using oil/fat samples spiked with a mixture of PCDD/Fs, DL- and NDL-PCBs as well as with PBDEs congeners. Sample intake was 2.5 gram. Results for all congeners were good, meaning recoveries all in the range of 85-100%.

Quite often also low fat containing samples have to be analyzed for these compounds and according to a report (3) published in 2014 by Hiroyuki Fujita from MIURA, the recoveries for PBDEs will be lower in case a low amount of fat/oil is put onto the set of columns when applying the standard purification method. This is caused by the polarity of PBDEs which is higher compared to the polarity of PCDD/Fs and PCBs resulting in a stronger adsorption onto the silica purification columns. In case samples with a lower amount of fat are analyzed a poor recovery of PBDEs was observed (4) while recoveries of PCDD/Fs and DL and NDL-PCBS were as expected all in the range of 80-100%

In order to improve the elution of PBDEs from the two silica columns, addition of a polar solvent e.g. Ethyl Acetate to the extract has been suggested (4). A disadvantage is that the amount of such a modifier depends strongly on the amount of oil/fat in the extracts. Too much modifier will result in low recoveries of the other compounds of interest. As an alternative to adding ethyl acetate, the volume of hexane eluting from the two silica columns was increased from 90 to 150 ml and in the presence of different amounts of oil/fat good recoveries could be obtained for compounds of interest including PBDEs.

Extraction

From each sample food, feed and environmental (soil, sediment) 5 to 10 gram is spiked with all relevant ^{13}C labelled congeners and after mixing with hydromatrix extracted with 100ml hexane/dichloromethane (1:1 v/v) (food) or with 100ml toluene/ethanol (3:7 v/v) plus 2% dodecane (feed/environmental). For low or non-fat containing samples 2% dodecane is added to the extraction solvent in order to act as a keeper

Each sample is transferred into a thimble and placed in the glass cups of the automated Solvent Extractor SER-158. The cups are filled via a dispenser with each 100ml solvent and the following five steps are automatically performed.

1. Immersion : Thimble with sample is placed in boiling solvent (60 min)
2. Removing : Solvent is evaporated until just below the thimble (10 min)[*1]
3. Washing : Classical hot soxhlet / twisselman extraction (60 min)
4. Recovery : Solvent is evaporated to near dryness (15 min) [*1]
5. Cooling : Glass cup is cooled (5 min)

To each cup 10-15 ml hexane is added as well as a clean-up standard e.g. ^{37}Cl-2,3,7,8-TCDD. Finally extracts are purified .

**1 In case of compound feed, minerals, soil or alike the extraction solvent should consist of a mixture of toluene ethanol (3:7 v/v) . Times for step 2 and 4 should be increased to respectively 15 and 30 minutes. As final extracts contains toluene a solvent exchange to hexane is necessary. Solvent transfer is done by adding 5 ml methanol to the extract. The formed azeotrope (Toluene/Methanol) with a boiling point of 65°C is again evaporated . The residue is dissolved in 10 ml hexane, $^{37}Cl_4$-2,3,7,8-TCDD (clean-up standard) is added and the extracts are purified.*

Purification of extracts

Clean-up is performed using an automated system (GO-xHT, Miura). For each extract a set of four in-line columns is required: silica gel impregnated with silver nitrate (1th); silica gel impregnated with sulfuric acid (2th); activated carbon (3th) and alumina (4th). The first and the second Column are used for purification of the extracts while the other two columns are used for trapping the compounds of interest. Extracts are transferred via a funnel to the first column ($AgNO_3$ Silica). Thereafter the set of columns and tubing is assembled and placed in the GO-xHT system. Column set is eluted with 150 ml of hexane with a flowrate of 2.5 ml minutes. In case only dioxins and pcbs are required the volume of hexane is only 90 ml. During this step the temperature of the two purification columns is maintained

at 60°C. The elevated temperature weakens the adsorption with silica gel and as a result the elution speed of dioxins and PCBs is enhanced. Also the chemical reaction rates (oxidation with sulfuric acid) with sample matrices is accelerated. PCDD/Fs and the four NO-PCBs are trapped on the activated carbon column while the MO, NDL-PCBs and PBDEs are trapped on the alumina column.
Finally in backflush, both the alumina and the carbon column are eluted using a small amount of toluene resulting in two fractions each of 1.5 ml. During these elution steps the temperature of the carbon and alumina column is set at 90°C. At first only the alumina column is eluted and the collected fraction contains the MO-PCBs, the NDL-PCBs and all PBDEs. After that the carbon column is eluted with toluene and this fraction contains all PCDD/Fs and NO-PCBs. To both fraction the recovery/syringe standards ^{13}C-1,2,3,4-TCDD and ^{13}C 2,3,4,6,7,8-HxCDF in 20 µl nonane is added and both fraction are concentrated to a final volume of 20 µl using an evaporator (CentriVap, Labconco).

GC-HRMS

The two obtained fractions are analysed using GC-HRMS (DFS High Resolution Magnetic Sector MS - Thermo Scientific) which is equipped with a PTV injector (Best P.T.V. injector) using a sintered glass liner (SGE pn 092155). For the determination of PCDD/Fs and the PCBs a VF-5ms 60m x 0,25mm x 0.25µm + 5m EZ-guard (Varian) GC column is used. For the determination of PBDEs a DB-5ms 15m x 0.25 mm x 0.10 µm GC columns is used. The mass spectrometer is operated in electron impact ionization mode, using selected-ion monitoring. From both fractions 4 µl is used to introduce the sample onto the GC. In figure 3 chromatograms are given of a procedure blank (whole method without matrix).

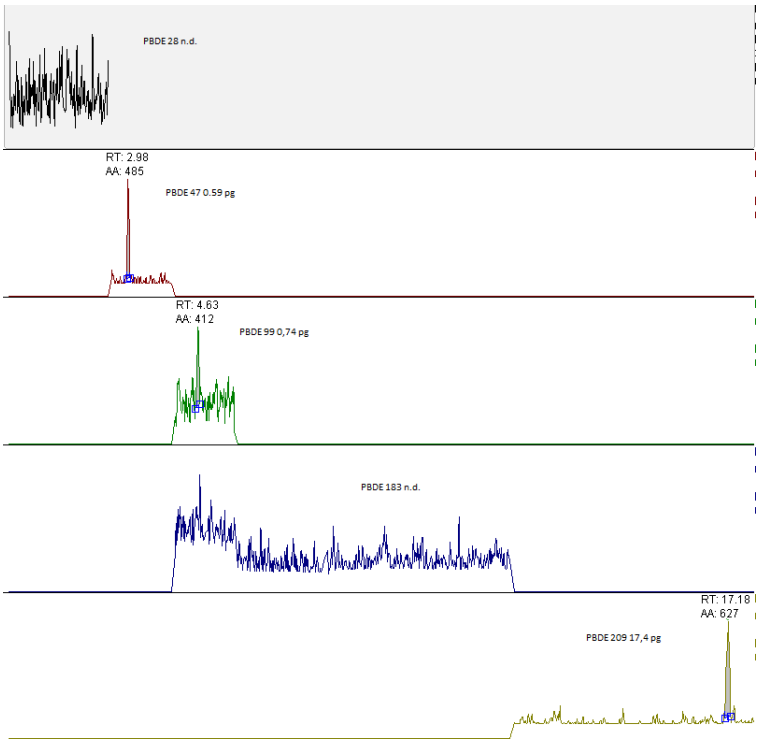

Fig 3: Chromatogram of procedure blank maximum amount of PBDE 209 is < 20 pg in final extract (20 ul), all other PBDEs less than 1 pg in final extract

Results and discussion

Advantages of the new approach are obvious; combination of short extraction times, use of small solvent volumes and high performance clean-up results in short delivery times and high quality chromatograms which are easy to process. Moreover, solvent exposure is minimized using closed systems which also contributes to lower background levels.

This automated system starts with a sample intake of up to 20 gram and the required volume of organic solvent is 70 up to 100 ml. Depending on the boiling point of the organic solvent the total extraction time required is approximately 2.5 hours. As the solvent is automatically concentrated down to near dryness the final extract fat/oil or dodecane can directly be put onto the Miura GO-xHT system. Purification using the Miura GO-xHT systems has many advantages such as: low solvent consumption 90ml hexane plus 4 ml toluene; Small final fractions only 1.5 ml toluene; no valves in sample pathway, no washing, no cross contamination and no additional clean-up is needed. Obtained extracts can be concentrated after transfer in a GC vial with tapered end which can be placed directly in an auto-sampler. Results in terms of recovery and quality of the chromatograms for PCDD/Fs and PCBs are similar to the results obtained with the standard MIURA method. So increasing of the volume hexane from 90 to 150 ml does not have any negative influence on the performance of the system for these congeners. For the determination of PBDEs the elution volume of hexane should be increased to 150 ml. By doing so excellent recoveries will be obtained. The method is applicable for all kind of matrices including vegetable oil, fish oil and fish.

The blank contribution from the chemicals and the equipment, including the Miura GO-xHT columns is very low. For PBDE 209 the sample extract contains no more than 20 pg. For the other PBDEs this is even a factor 20 lower.

By using the SER 158 extraction systems, a GO-6HT purification system and a Centrivap concentrator, one technician can perform the complete sample preparation procedure of more than 100 Food and/or Feed samples in one week time.

References

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