SMART and Simultaneous Automated Clean-up of PCBs, PCDD/Fs, and PBDEs in Environmental Samples

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LCTech

Content

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1. Introduction

Persistent organic pollutants (POPs) are well known in relation to food topics. Especially when it comes to "dioxins", more precisely polychlorinated dibenzo-para-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs), scandals referring to contaminated eggs, meat, and diary products brought public interest in the past. The reason is rather simple: up to 90 % of the human uptake of these highly toxic, lipophilic, bioaccumulative substances derive from food. Thus, attention and awareness as well as legal frameworks, boundery values and analytical approaches are globally well established. European examples are Directive 2002/32/EC, Commission Regulation (EC) No 1881/2006, Commission Recommendation 2011/516/EU and references therein.

However, it is obvious that food is not the original source of POP release yet only an intermediate storage and conveyor of these hazardous substances into the human body as terminal end of the food chain. The production of POPs is mostly bound to industrial processes, either intentional fabrication (e.g. PCBs) or unintentional formation due to incineration (e.g. PCDDs). Although there are plenty of filtration techniques, exhaust gases are still a major pathway of POP release. Subsequentely, the aeolian transport of this so-called atmospheric particulate matter, which has been recognized as the major environmental airborne pollutant, causes severe impact at a global health scale, i.e. ecosystems and human health.

The fact that plenty of airborne particles can be hardly photodegraded and able to withstand atmospheric degradation is also worrisome. This increases their atmospheric residence time and inevitably leads to long-range atmospheric transport (LRAT), resulting in exposure shifts to areas which are far from any contaminating source. The same accounts for polybrominated diphenylethers (PBDEs), which have been globally used as flame retardants. The commercial formulations of penta-, octa- and deca-BDEs were extensively used in the past and in turn, BDEs such as BDE-47, -99, -183, and -209 got released into the environment in large scales, becoming ubiquitous, and having, for instance, been found in significant amounts in human breastmilk already (see LCTech application note). Together with PCBs and PCDD/Fs, PBDEs are today regulated by the Stockholm Convention.

Although all these substances are officially banned in most countries, there is a constant need for characterizing and quantifying the baseline levels and daily spreading. Compared to the food and feed sector, only few regulations and normatives are available (e.g. EPA Method 1613b). As the punctual risk of intoxication for the individual human is lower due to by far lower environmental concentrations, it has not been too much in the focus of global policy makers. Furthermore, the analytical challenges regarding overall extremely low analyte concentrations as well as heavy, yet, highly diverse matrix loads allowed only few specialized labs to work on this topic. In this study, air samples were tested to validate an automated DEXTech method for the simutaneuos clean up of PCBs, PCDD/Fs and PBDEs. Comprehensive sample preparation is necessary to achieve reliable results and satisfying recoveries measuring dioxins, PCBs and PBDEs down to the femtogram (fg) level.

2. Method Development

All blanks and samples were prepared in a final volume of 10 mL for their injection into the DEXTech Plus system. To study the optimal performance, three different approaches where applied.

- Blanks of n-hexane and air samples without adding toluene.
- Blanks applied with 20% of toluene as a modifier (as instructed by LCTech).
- Samples with up to 10 % of dichloromethane or 10 % and 5% of acetone.

2.1 Reagents and Materials

- DEXTech Plus automated sample clean-up device (LCTech GmbH, Obertaufkirchen, Germany)
- Universal Columns for dioxin analysis, alumina clean-up columns and carbon clean-up columns (LCTech, Obertaufkirchen, Germany)
- SMART columns for dioxin analysis, alumina clean-up columns and carbon clean-up columns (LCTech, Obertaufkirchen, Germany)
- Magnetic Sector HRGC-HRMS system (DFS, Thermo Fisher Scientific, Bremen, Germany).
- Specifically, 13C12-labeled standards (solutions P48-M-ES, P48-W-ES, MBDE-MXG, and EN1948-ES from Wellington laboratories, Guelph, ON, Canada) corresponded to forty-four POPs:
  - Eighteen PCBs
    - 6 indicator (i-PCBs) #28, 51, 101, 138, 153, 180
    - 12 dioxin-like (DL-PCBs): 4 non-ortho (#77, 81, 126, 169) and 8 mono-ortho (#105, 114, 118, 123, 156, 157, 167, 189)
  - Thirteen BDEs #3, 15, 28, 47, 99, 100, 126, 153, 154, 183, 197, 207, 209
  - Thirteen PCDD/Fs (all 17 2,3,7,8-sustituted congeners for toxicity assessment except the following four):
    - 1,2,3,7,8,9-hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin;
    - 1,2,3,7,8-pentachloro[$^{13}C_{12}$]dibenzofuran;
    - 1,2,3,7,8,9-hexachloro[$^{13}C_{12}$]dibenzofuran; and
    - 1,2,3,4,7,8,9-heptachloro[$^{13}C_{12}$]dibenzofuran

Figure 1: SMART, column adapters, Aluminium oxide, and Carbon column as used for the experiments

2.2 Sample Preparation and Clean-up

The samples were collected on polyurethane foams (PUFs). These were spiked with a suite of PCDD/F, PCB, and PBDE 13C12-labeled standards prior to Soxhlet extraction (24 h) with an n-hexane/dichloromethane mixture (9:1). The extracts were evaporated and automatically cleaned-up by using the Alumina Plus method on a DEXTech Plus (figure 2) as described below. The Alumina Plus method can be also used on a DEXTech Pure, DEXTech Heat or DEXTech 16.

The automated clean-up method was originally developed to analyse polychlorinated dioxins and furans (PCDD/Fs) as well as polychlorinated biphenyls (PCBs) in food and feed. Besides mono-ortho- and i-PCBs, PBDEs are eluted in fraction 1 by a n-hexane/dichloro methane-mixture (1/1, v/v) as well as fraction 2 non-ortho PCBs and PCDD/Fs in toluene (figure 3). Final extracts were rota-evaporated to ~ 1 mL, transferred to vials, and dried under a gentle nitrogen steam. Fractions were reconstituted in a few microliters of the respective PCDD/F, PCB, and PBDE 13C12-labeled injection standards prior to GC-HRMS-analysis.

Figure 2: Automated clean-up system DEXTech Plus

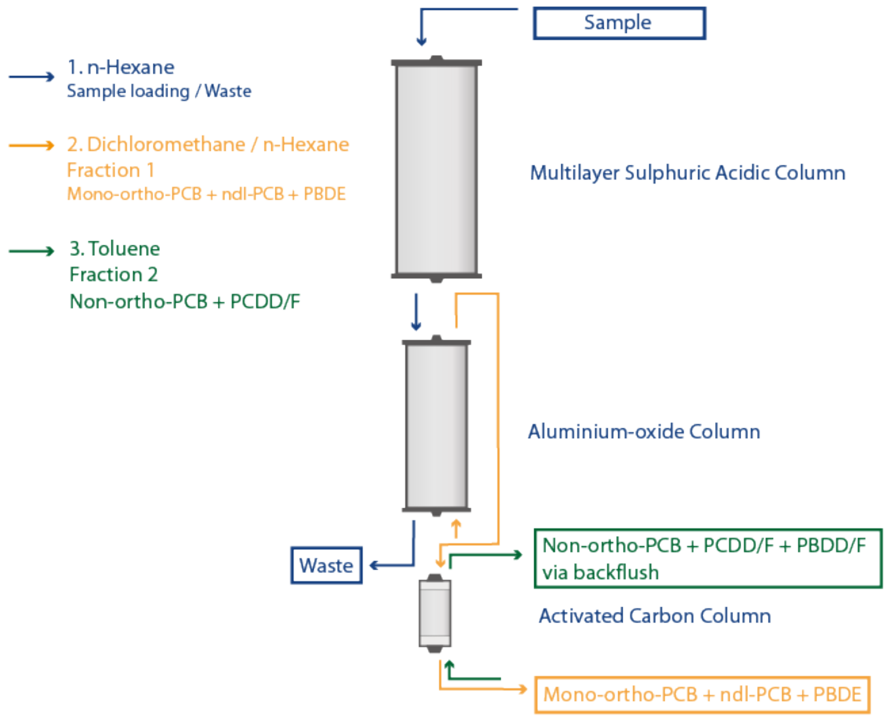

Figure 3: Flow path of the Alumina Plus Method

The sample is loaded with n-hexane on the aluminium oxide column after lipid degradation on the acidic silica column. Degradation products go to waste while target compounds are trapped on the aluminium oxide column. Mono-ortho-, i-PCBs, and PBDEs are separated from the planar compounds and collected as fraction 1 by a 1:1 mixture of dichloromethane and n-hexane. PCDD/Fs and coplanar-PCBs are retained on the top of the carbon column and collected in backflush elution with toluene.

2.2.1 Software Protocol

DEXTech Plus provides two default methods for the aluminium oxide column, one using the Universal column (for samples up to 5 g fat content) the second using the SMART column (for samples with <1.5 g fat content). Additionaly the user can freely parameterise methods and save them in the system.

In this case, the default method using the **SMART** column (column 1) together with the **Aluminium oxide** column (column 2) and a **Carbon** column (column 3).

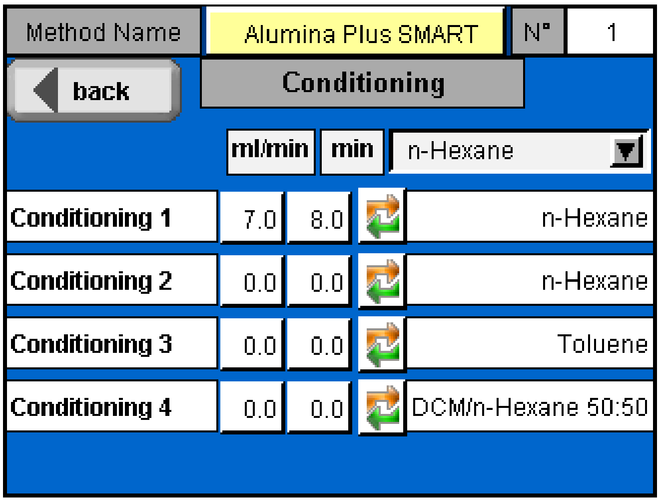

Figure 4: Parameters for the conditioning steps (Alumina Plus SMART Method)

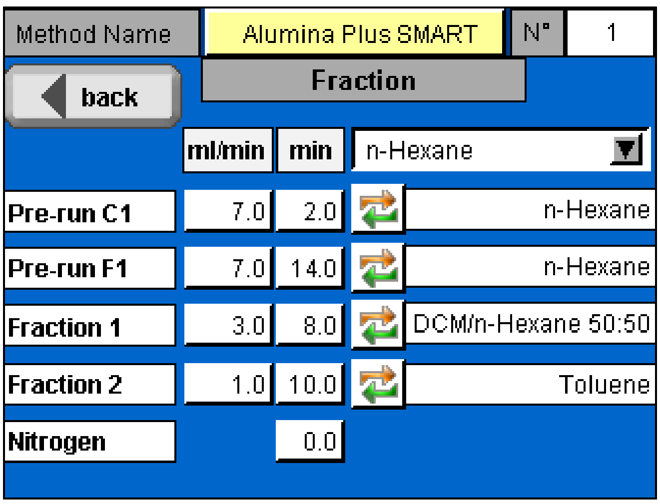

Figure 5: Parameters for the fractioning (Alumina Plus SMART Method)

2.2.2 Fractions, Process, and Solvent Management Parameters

Fractionation Alumina Plus SMART method:

Fraction 1:	i-PCBs, mono-ortho-PCBs, PBDEs
Fraction 2:	non-ortho-PCBs, PCDD/Fs

Process time: 45 min

The process time includes the loading of 10 mL sample as well as the rinsing of the sample vial with 3 × 1 mL solvent (Fig. 6).

Solvent consumption:

n-Hexane:	168 mL
n-Hexane /DCM:	24 mL
Toluene:	10 mL
Total:	202 mL

Figure 6: Automated rinsing of sample vial

2.3 Instrumentation

Analysis was performed by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) with a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany).

GC-MS-Setup

- **Injection temperature: 260 °C**
- **Injection volume: 1 µL (splitless mode)**

GC columns

- **PCB and PCDD/F separation: DB-5MS column (Agilent J&W, USA) 60 m × 0.25 mm × 0.25 µm**
- **BDE separation: Rxi-5Sil MS column (Restek, USA) 15 m × 0.25 mm × 0.10 µm**

Different oven temperature programs were used for each family of analytes. Positive electron ionization (EI+) was used operating in selected ion monitoring (SIM) mode at 10.000 resolving power (10% valley).

3. Results

The major result was to find the optimal standard procedure for joint automated clean-up of PCDD/Fs, PCBs and PBDEs via DEXTech Plus. Table 1 shows an overview of the tested solvent modifiers and the subsequent judgment of the POP class recoveries using Universal, Aluminium oxide and carbon columns.

Table 1 POP classes and solvent modifiers

	Recoveries		
Solvent modifier	**PCBs**	**PCDD/Fs**	**PBDEs**
Toluene (20%)	good	good	good (BDE-209>50%)
Dichlormethane (10%)	**fair**	**good**	**very low**
Acetone (10 %)	good	good	good (BDE-209≈70%)

The results from above were transferred to an exemplary application with air samples. For the clean-up procedure on DEXTech Plus, a setup of SMART, aluminium oxide and carbon column was used as described in the methods section. Due to composition differences in SMART and Universal columns, only 5 % of acetone had to be added as solvent modifier for optimal results.

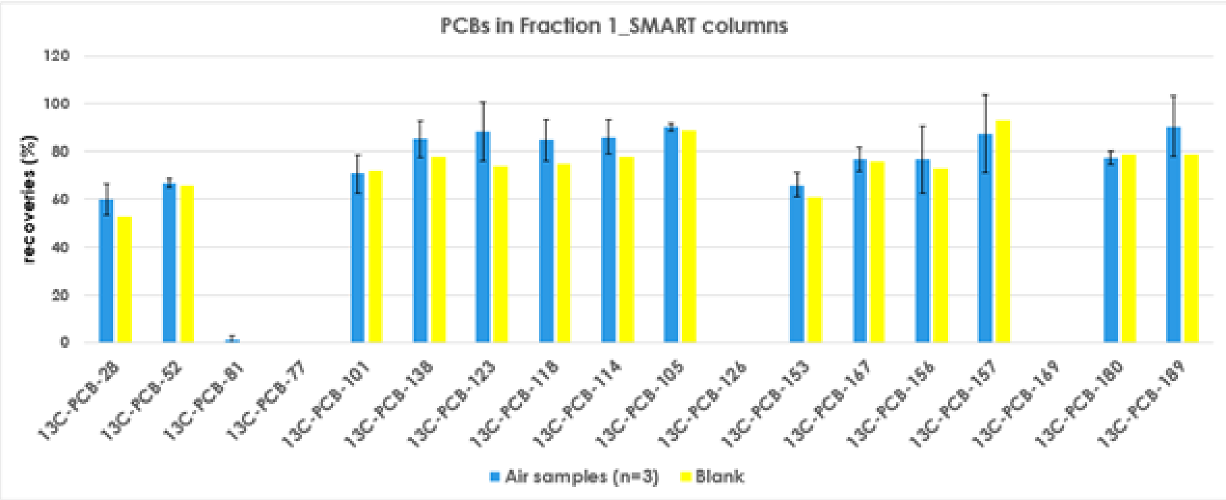

Figure 7: Percentage recoveries of mono-ortho and ndl-PCBs in DEXTech Plus fraction 1

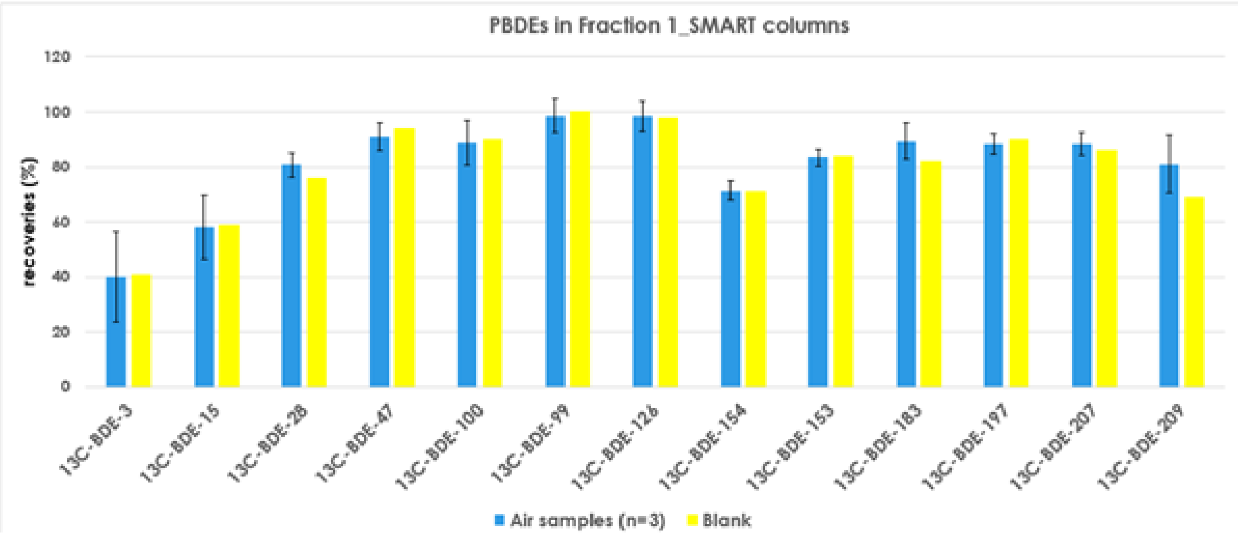

Figure 8: Percentage recoveries of PBDEs (right) in DEXTech Plus fraction 1

The comparison of air samples and blanks (both spiked with 13C12-labeled standards) shows excellent clean-up results with DEXTech Plus. The recoveries of any congener are the same for samples and blanks even at difficult analytes like BDE-209. Only the low chlorinated BDE-3 is below the 60 % recovery line. A loss of analyte at the evaporation or in other steps of sample preparation is likely.

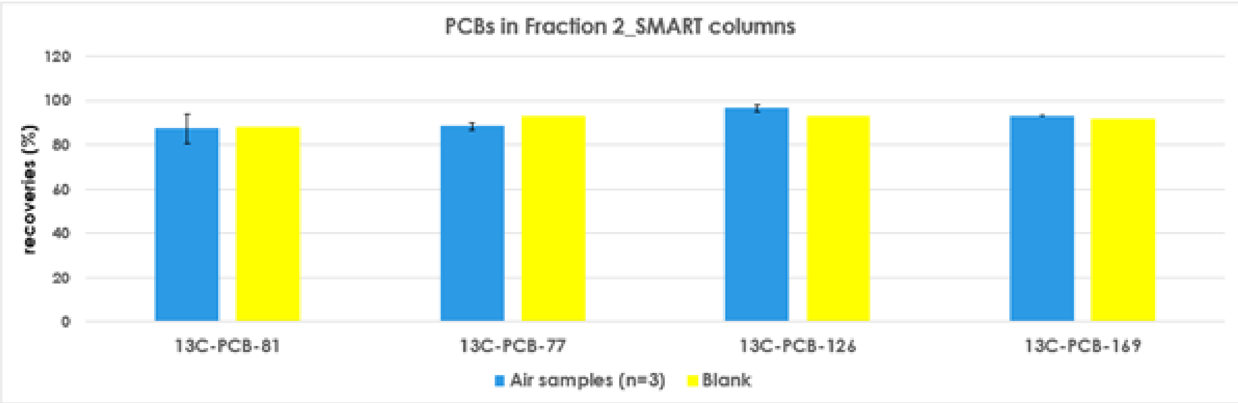

Figure 9: Percentage recoveries of non-ortho PCBs in DEXTech Plus fraction 2

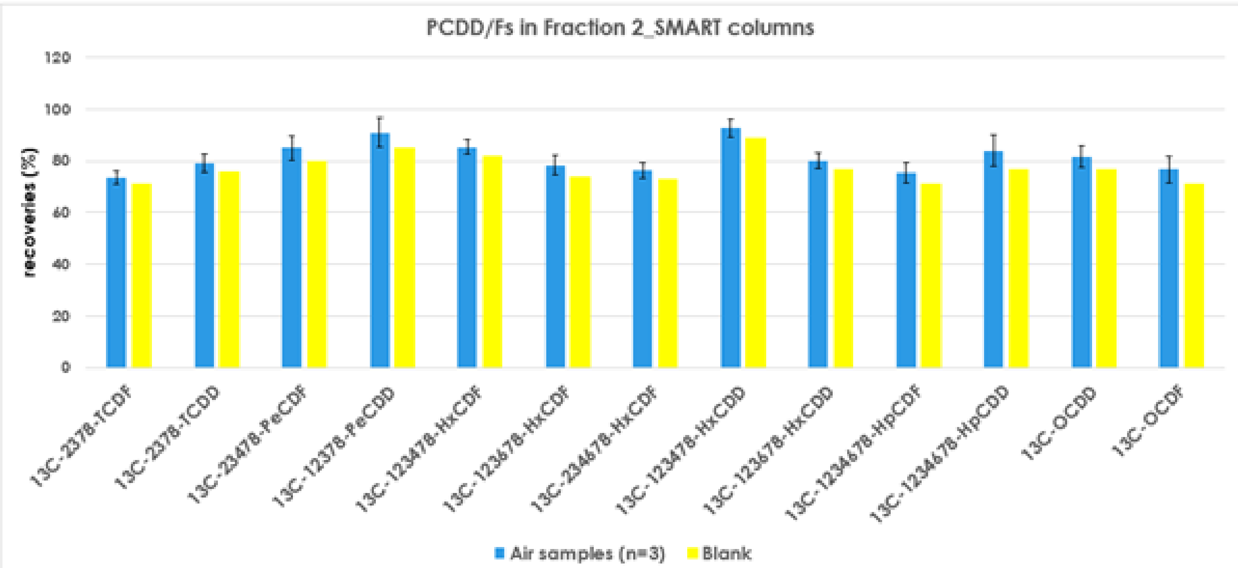

Figure 10: Percentage recoveries of PCDD/Fs (right) in DEXTech Plus fraction 2

Fraction 2 leads to outstanding results as well. All congeners were recovered at ideal ranges in samples and blanks. This strongly underlines the dedication of DEXTech Plus to excellent dioxin clean-up.

Due to the recovery results showed above, the clean-up using the SMART column approach with 5 % of acetone modifier was applied to the certified Standard Reference Material (SRM) 1945 “Organics in Whale Blubber” (United States Department of Commerce, National Institute of Standards and Technology, Gaithersburg, Maryland, US).

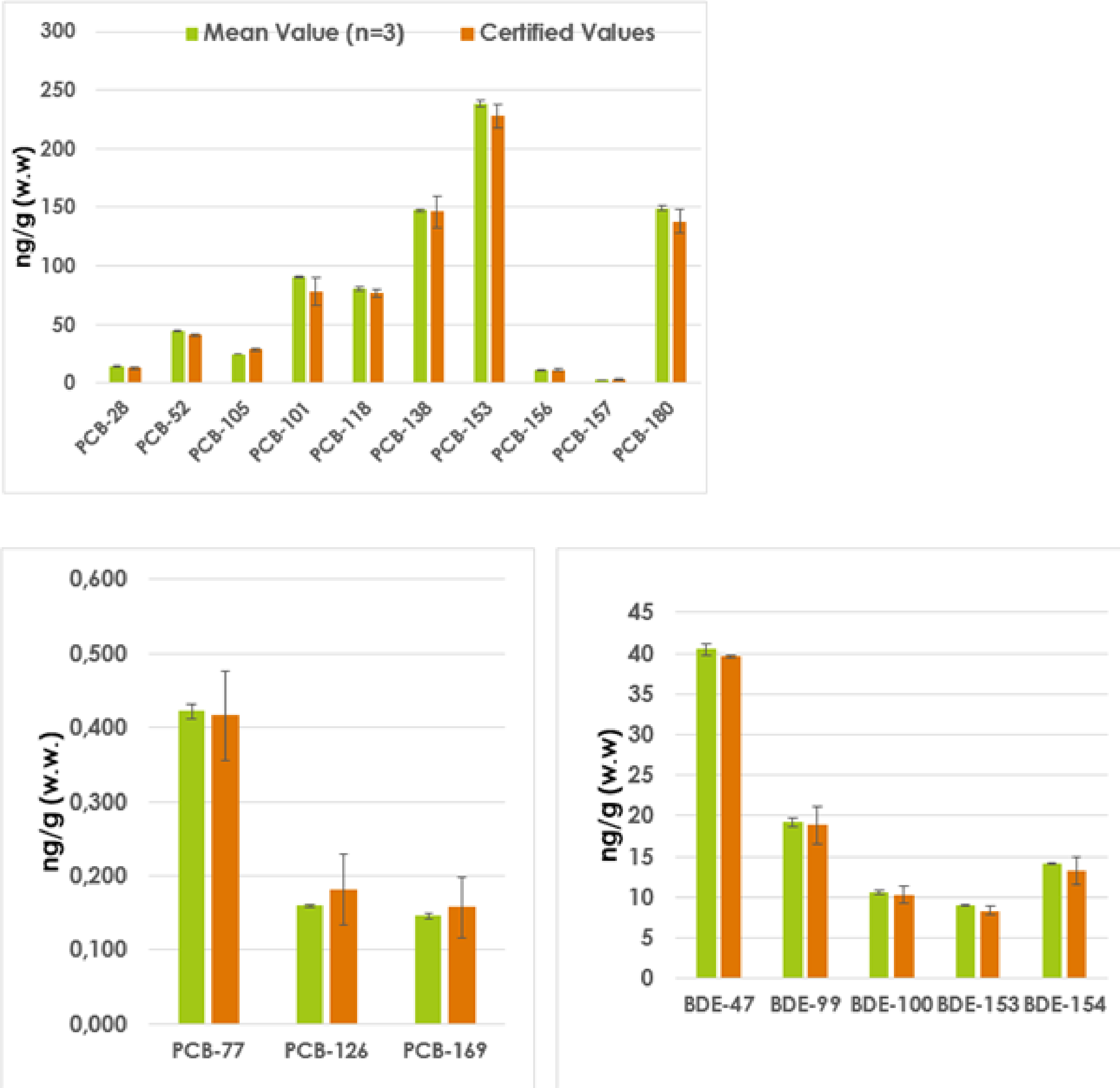

Figure 11: Comparison of single congeners from processed SRM to assigned certified values in ng analytes per g sample weight. Evaluation of mono-ortho and ndl-PCBs (above), non-ortho PCBs (down, left) and PBDEs (down, right) after DEXTech Plus clean-up.

Excellent agreement between experimental results and certified values from SRM1945 is shown in figure 11. This shows, that not only overall recoveries of intra-laboratory quality standards are great. Moreover, the setup of DEXTech Plus using SMART, Aluminium oxide, and carbon colums leads to outstanding results at global round robin tests and make it a highly reliable “must have” in environmental PCB, PCDD/F and PBDE clean-up.

4. Further Studies

As shown above, the Alumina Plus method performed on the DEXTech Plus instruments is a fast and reliable method for the simultaneous clean up of PCBs, PCDD/Fs and PBDEs. Since the validation studies, the method has been used in our lab for a variety of studies with the most different matrices as listed below:

- Different marine species like plankton and a variety of fish (tuna, hake, mackerel) within a Mediterranean trophic web (Castro-Jiménez et al., 2021)
- Antarctic penguin eggs (Morales et al.,2022)
- Blubber of sperm whales and dolphins from the Mediterranean Sea (Bartalini et al., 2018 and Capanni et al., 2020).
- Blubber of Mediterranean Cuvier's beaked whales (Baini et al., 2020)

5. Acknowledgement

All the tests in this application note were done at the Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC, Madrid, Spain. We thank Juan Muñoz-Arnanz and Begoña Jiménez for generously providing the data for this application note.

6. Related Information and References

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7. Annex

Instruments

1.	DEXTech Plus	P/N	15011
2.	DEXTech Pure	P/N	15969
3.	DEXTech Heat	P/N	16800
4.	DEXTech 16	P/N	15430

Clean-up columns

1.	Universal column	P/N	19511
2.	SMART columns	P/N	19513
3.	Alox column	P/N	15433
4.	Carbon column	P/N	15242

Any Questions?
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