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Automated analysis of pcdd/fs, dioxin-like pcbs, non-dioxin-like pcbs and polybrominated diphenyl ethers in food and feed

Abstract

Polychlorinated dibenzo-p-dioxin (PCDD/Fs) and polychlorinated biphenyls (PCBs), dioxin-like and non-dioxin-like have been subject of many incidents with food and/or feed (1). At the end of 2010, the use of industrial grade fatty acids for the production of feed caused a major incident in Germany (2)
As a result of these incidents for some matrices a positive release is demanded (3,4), resulting in a request for increased sample throughput and shorter turn around times. Using comprehensive automation and new technology, sample capacity has been increased. Nowadays to well over 100 samples per week can be handled by one technician, with a turnaround time of eight hours to two days. At the same time the required labor per sample has reduced resulting in a capacity of 1500-2000 samples per FTE at a lower overall cost. As the interest in the determination of flame retardants is growing, the method for the determination of Dioxins and PCBs has been extended to simultaneously analyze Poly Brominated Diphenyl Ethers (PBDEs).

Introduction

The detection of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (non-ortho, mono-ortho substituted CBs) as well as NDL-PCBs in food, feed and environmental samples has been subject of intensive research. Concentrations in biological samples are very low, in general in the low pg/g range while in environmental samples the concentrations are somewhat higher. In order to achieve low limits of quantitation LOQs, highly sensitive and specific methods are required. Prior to extraction ^{13}C labelled (internal standards) are added to the samples for identification and quantification purposes. In general methods are set up in several steps. Extraction is often done by classical soxhlet and the obtained extracts are purified by an array of columns. Frequently used adsorbents are : Silica (acidified) , Alumina, Florisil, Carbon etc.

One 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. a Carbon or Florisil column. The 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 since 2014 with GC-MS-MS, In the early days, the capacity of commonly used methods was limited 20-30 samples per week with a turn around time of one to two weeks. Especially during a crisis when results are urgently required for decision making, there has been a strong demand for faster methods.

In order to improve extraction and purification methods in terms of capacity and turnaround time while at the same time maintaining the quality, the focus has been on automation since 2000.
In 2015 a Japanese company "MIURA" introduced via DSP-Systems in Europe a new technology (5,6) with the following advantages:

- Low solvent consumption (100ml per sample)
- Faster (concentration of 1.5 ml extracts only)
- Easy connection of columns
- Excellent clean-up due to high performance adsorbents and heated zones
- Unique way of flow switching without valves
- No cross contamination

This systems is applicable for the determination of PCDD/Fs, DL-PCBs and NDL-PCBs. Since 2015 this clean-up technology is being used in routine by more than 10 European laboratories. Recently the program of the purification system has been slightly modified in order to be able to purify sample extracts for PBDEs simultaneously with Dioxins and PCBs. PBDEs are quantitatively collected in the same fraction as the MO and NDL-PCBs .

PBDEs

Since the last decade the demand for methods for the simultaneous analysis of PCDDs/PCDFs, PCBs, and PBDEs in environmental, food and feed samples has increased. To investigate if the MIURA system is also applicable for the purification of PBDEs the standard purification method was tested using oil/fat samples. Sunflower oil was spiked with ^{13}C labeled PCDD/Fs, DL- and NDL-PCBs as well as with seven ^{13}C labeled PBDEs congeners (28, 47, 99, 153,154, 183 and 209). 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 samples have to be analyzed for these compounds and according to a report (7) 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 phenomena 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. To investigate the influence of the oil/fat amount in an extract on the PBDE recoveries, different amounts of sunflower oil were purified twice after spiking with all compounds of interest. In figure 1 the results of these experiments are shown proving indeed that the recovery of PBDEs strongly depends on the amount of fat/oil introduced onto the purification column when using the standard program. Recoveries of PCDD/Fs and DL and NDL-PCBS were as expected all in the range of 80-100%

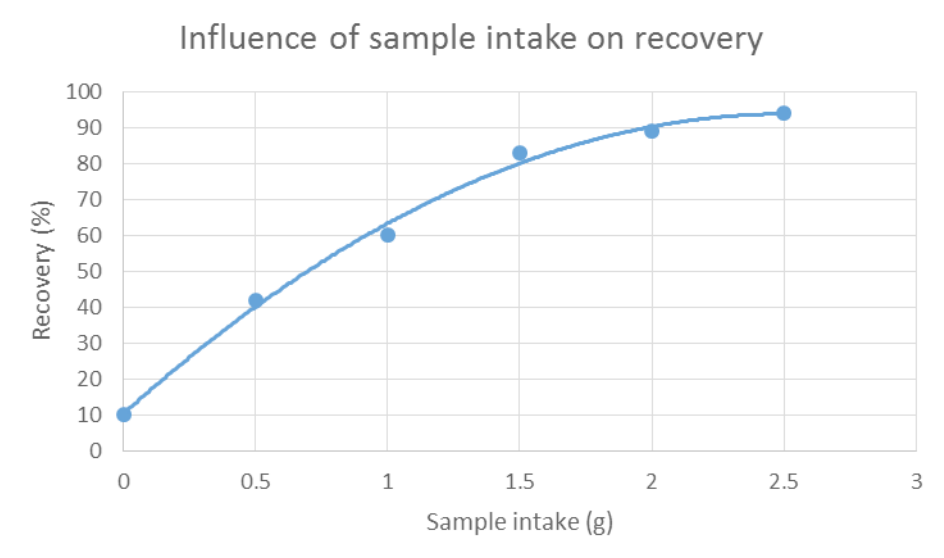


Figure 1: Recovery of PBDEs in relation to sample intake

In order to improve the elution from the two silica columns Hiroyuki Fujita suggested the addition of a polar solvent e.g. Ethyl Acetate to the extract. However the amount of modifier which should be added to the extracts depends strongly on the amount of oil/fat in the final extracts. Too much modifier will result in low recoveries of the other compounds of interest.

Although the addition of modifier works quite well in case the exact amount of fat is known, this critical step should be avoided if possible. To overcome this critical step a number of experiments were performed with the aim to describe a generic method which is applicable for all kind of matrices independent of the oil/fat content.

As an alternative to using a modifier we tested if increasing the volume of hexane for the elution of all compounds of interest from the silica columns onto the concentration column could give good results. The volume of hexane was increased from 90 to 150 ml and again different amounts of sunflower oil were purified twice after spiking with all compounds of interest. In table 1 the recovery results of PBDEs are given.

Results of recovery experiments using modified MIURA program with different sample intake

	intake (g)	intake (g)	intake (g)	intake (g)	intake (g)	intake (g)	intake (g)
	0.0	0.5	0.5	1.0	1.0	2.5	2.5
	Rec%	Rec%	Rec%	Rec%	Rec%	Rec%	Rec%
PBDE 28	86	88	96	82	101	95	93
PBDE 47	103	85	102	86	107	100	98
PBDE 99	107	104	118	97	102	102	96
PBDE 153	82	91	77	79	95	106	94
PBDE 154	89	86	102	72	96	96	85
PBDE 183	79	95	115	100	106	110	101
PBDE 209	74	99	97	89	106	102	96
Avg (%)	88.6	92.6	101.0	86.4	101.9	101.6	94.7
Stdev (%)	12.26	7.09	13.56	9.88	4.88	5.29	5.02

Table 1: Recoveries of all congeners in spiked sunflower oil with different sample intake using the modified program
Recoveries for the PCDD/Fs , DL and NDL-PCB are given in Annex 1. From table 1 it is clear that all recoveries are within the expected range and independent of sample intake.

Materials and methods

Extraction

With exception of oil/fat samples, which are directly diluted after spiking with all relevant ^{13}C labelled congeners, samples are extracted using e.g.:

1. Pressurised Liquid Extraction (Speed Extractor Buchi)
2. Automated Solvent Extractor based on Randal principle (SER-158 Velp Scientifica)

Ad 1. After spiking with all relevant ^{13}C labelled congeners, Food and Feed samples, including fish and derived products, are extracted with hexane/dichloromethane (1:1, v/v) at 100°C and 1500 PSI during 10 minutes in three cycles, total extraction time is 45 minutes. If necessary samples are first freeze-dried or mixed with hydromatrix (preferred) to get a free flowing powder. For compound feed the extraction is performed with toluene/ethanol (9:1, v/v), All extracts obtained after extraction with hexane/dichloromethane are concentrated down to 5-10 ml using a rotary evaporator (KNF ROTAVAP RC 600). After addition of the ^{37}Cl-2,3,7,8-TCDD (clean-up standard) the final volume is made up to approximately 10 ml with hexane. Finally extracts are purified .

All extracts obtained after extraction with toluene/ethanol are concentrated down to < 1.0 ml using a rotary evaporator. In order to get rid of any traces of toluene a solvent transfer is done by adding methanol to the toluene extract as well as 100-200ul dodecane as keeper. 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-2,3,7,8-TCDD (clean-up standard) is added and the extracts are purified.

Ad 2. After spiking with all relevant ^{13}C labelled congeners, Food and Feed samples are mixed with hydromatix and 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 80 ml Hexane/DCM (1:1 v/v)*1 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 (30 min)
3. Washing : Classical hot soxhlet extraction (30 min)
4. Recovery : Solvent is evaporated to a volume of ≈ 5-10 ml (15 min)
5. Cooling : Glass cup is cooled (5 min)

Final extracts contains each 5-10 ml hexane. After addition of the ^{37}Cl-2,3,7,8-TCDD (clean-up standard) the final volume is made up to approximately 10 ml with hexane. Finally extracts are purified .

*1 In case of compound feed or minerals the extraction solvent should consist of a mixture of toluene ethanol (3:7 v/v) . Times for step 2 and 4 should be increased. As final extracts contains toluene a solvent exchange to hexane is necessary

Purification of extracts

Clean-up is performed using the new purification technology (GO-xHT, Miura), which was introduced in Europe in 2015 - Figure 2A and 2B. For each extract a set of four in-line columns is required: silica gel impregnated with silver nitrate (1); silica gel impregnated with sulfuric acid (2); activated carbon (3) and alumina (4). Column 1 and 2 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, figure 2B. Column set is eluted with

150 ml of hexane with a flowrate of 2.5 ml minutes. 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 or nitric 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).

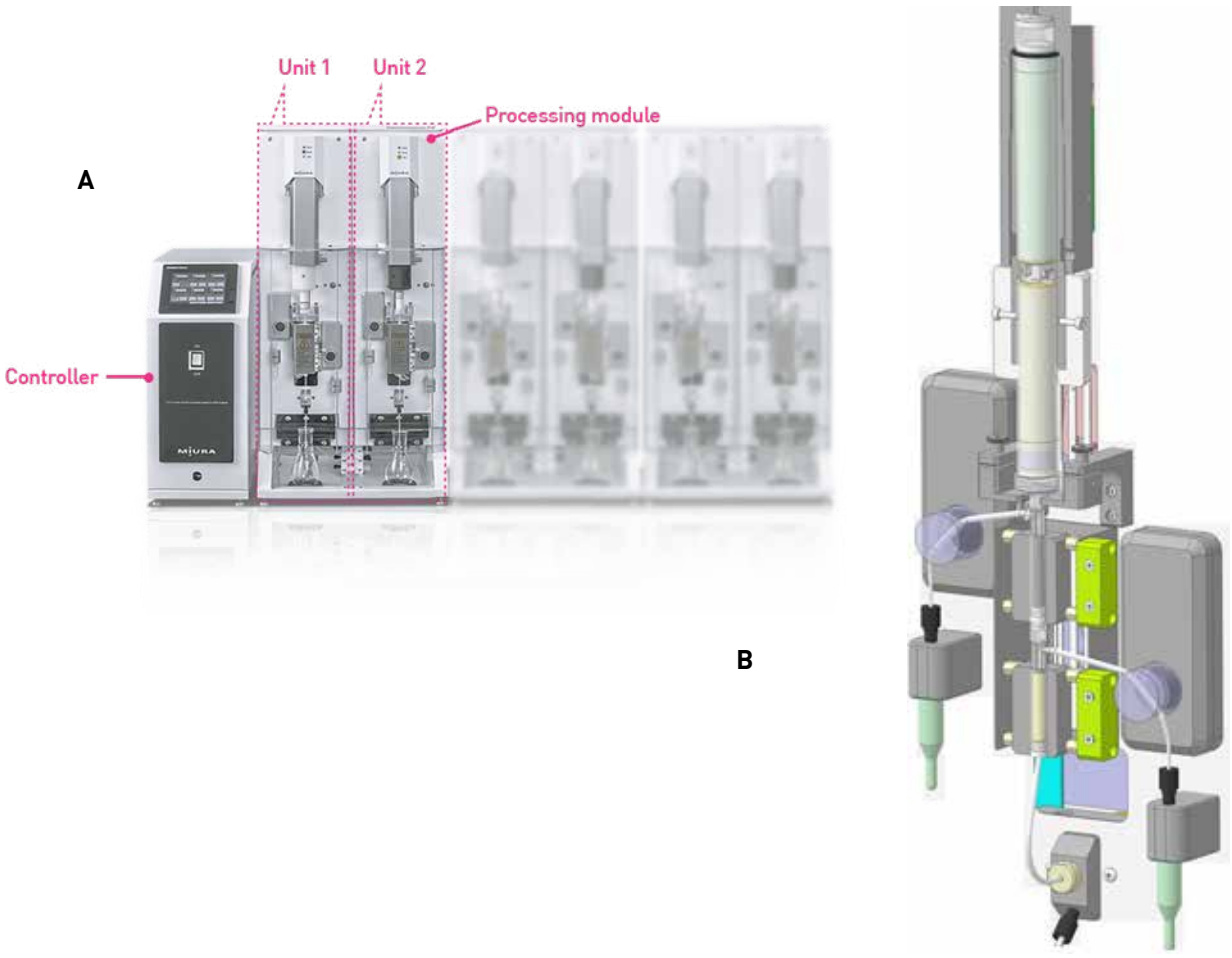


Figure 2: **A** Set up of the GO-xHT system capable of simultaneous purifying 2, 4 or 6 samples within 90 minutes. **B** Schematic diagram of the column flow channel of the GO-xHT system

GC-HRMS

The two obtained fractions are analysed using GC-HRMS (DFS High Resolution Magnetic Sector MS - Thermo Scientific) each MS is equipped with two GCs each 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. After data reduction, results are directly transferred to a Laboratory Information Management System (LIMS) and after approval reported to the customers.

In figure 3, 4 and 5 chromatograms are given of respectively a standard mixture, a sample oil spiked with PBDE 209 and a procedure blank (whole method without matrix).

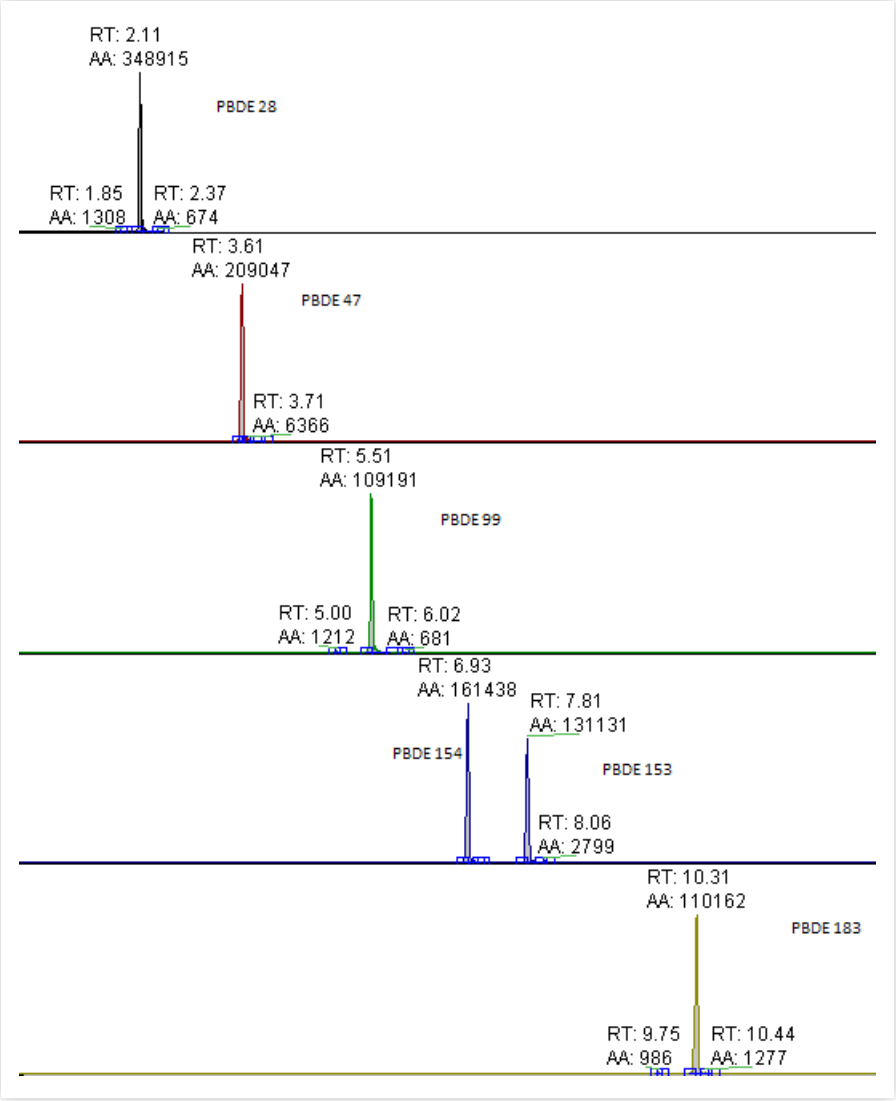
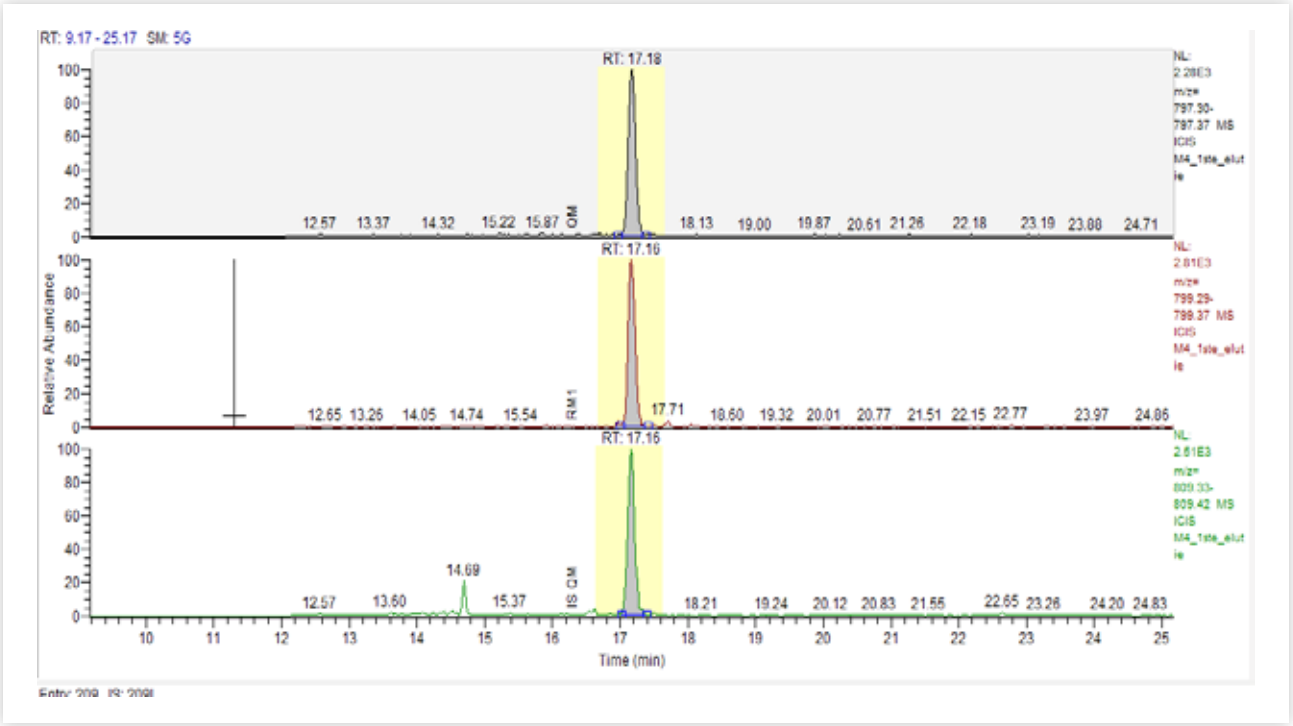


Fig 3: Chromatogram of standard mix containing PBDE 28; 47; 99; 154; 153; 183

A



B

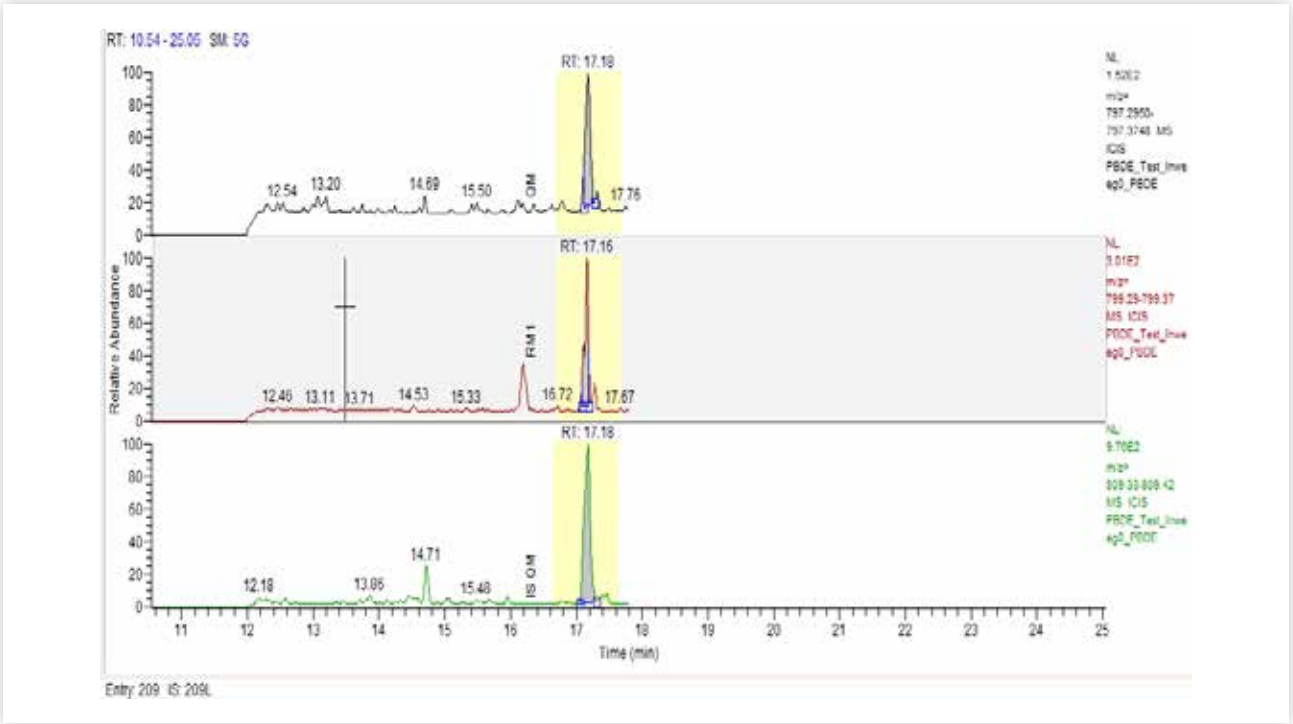


Fig 4: **A** Chromatogram of sample vegetable oil spiked with PBDEs 40 pg/gram, **B** Chromatogram is blank vegetable sample

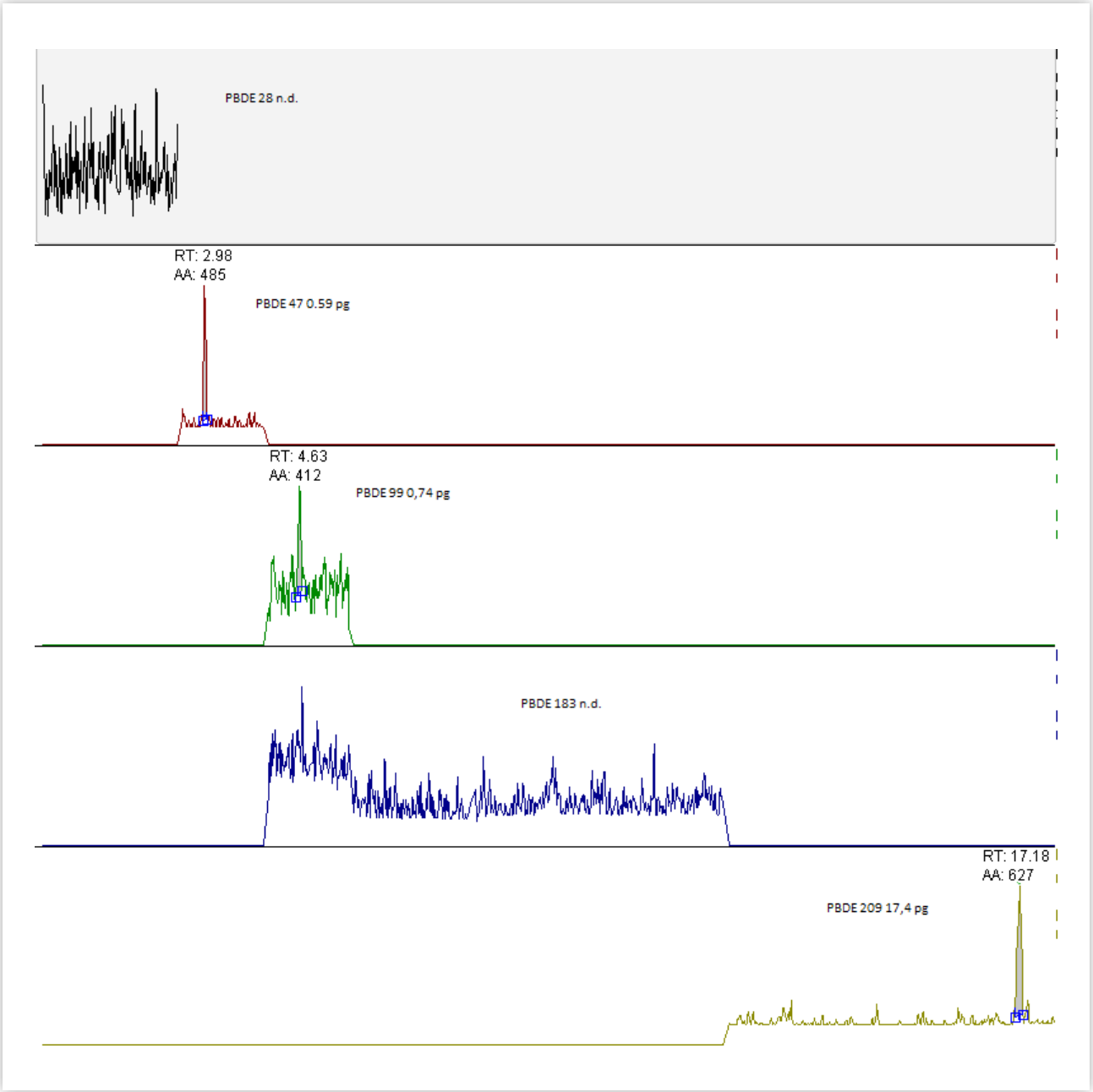


Fig 5: Chromatogram of procedure blank maximum amount of PBDE 209 is < 20 pg in final extract all other PBDES less than 1 pg in final extract

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. The use of accelerated solvent extraction compared to classical soxhlet extraction benefits in speed of extraction using less solvent and less bench space. In order to have at the same time a number of extracts - e.g. six - ready for purification, a system capable of parallel extraction is preferred. Although solvent consumption is low still a final volume of 50-70 ml will be obtained so additional concentration using e.g. a rotary evaporator is needed. As an alternative the new fully automated soxhlet technique "SER 158", based on Randall principles (8) can be used. 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 5-10 ml the final extract 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 fraction
  - First fraction: MO-PCBs, NDL-PCBs and PBDEs in ≈ 1.5 ml toluene
  - Second fraction : PCDD/Fs and NO-PCBs in ≈ 1.5 ml toluene
- Run time of only 90 minutes
- No valves in sample pathway, no washing, no cross contamination, no conditioning
- No additional clean-up needed
- Excellent recoveries
- High quality chromatography
- Uncomplicated, ready-to-use

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. When started with a sample intake of 2.5 gram this mean a blank contribution of less than 8pg/gram. For the other PBDEs it 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

1. Ron Hoogenboom , Wim Traag, Alwyn Fernandes and Martin Rose ; European developments following incidents with dioxins and PCBs in the food and feed chain. Food Control 50 (2015) 670-683

2. Abraham, K., Appel, K. E., Berg, K., Heinemeyer, G., Lahrssen-Wiederholt, M.,Large, N., et al. (2011). Review: die vorkommnisse um dioxin in futtermitteln in Deutschland 2011-gab es ein risiko für verbraucher? Journal of Food Safety and Food Quality, 62, 105-144.

3. EU-legislation, as laid down in Reg. (EU) No. 225/2012, in compliance with Commission Regulation (EC) No 152/2009.

4. GMP+ Feed Certificate scheme; GMP+ BA4, Minimum requirements for Sampling and

5. Hiroyuki Fujita, Noriaki Hamada, Kazuyuki Sawadaishi, Katsuhisa Honda, Development of a sample preparation system for Dioxins: application to immunoassay, Organohalogen compounds- Vol.66, pp.677 (2007)

6. Marchand P, Lesquin E, Brosseaud A. , Vaccher V, Vénisseau A, Le Bizec B, A new and highly innovative automatic purification system evaluated for dioxins and PCBs, Organohalogen Compounds Vol. 76, 546-549 (2014)

7. Hiroyuki FUJITA, Takanori Makino, Kenji Inaba, Kazuki Yamamoto, Simultaneous analysis for DIOXINS, PCBs and PBDEs with a fully automated sample preparation system, PAO020348, Dioxin 2015 (see http://dspsystems.eu/wp-content/uploads/2016/02/SIMULTANEOUS-ANALYSIS-FOR-DIOXINS-PCBS-AND-PBDES.pdf)

8. Eljarrat E, Caixach J, Rivera J., Extraction of polychlorinated dibenzo-p-dioxins and dibenzofurans from solid samples using the Randall technique., Chemosphere. 2000 Jan;40(2):187-93.

Annex 1: Recovery of all congeners with varying sample intake

Results of recovery experiments using modified MIURA method with different sample intake							
	Intake (g)	Intake (g)	Intake (g)	Intake (g)	Intake (g)	Intake (g)	Intake (g)
	0	0.5	0.5	1	1	2.5	2.5
2,3,7,8-TCDD	80	78	83	83	88	85	72
1,2,3,7,8-PeCDD	102	93	93	91	91	96	84
1,2,3,4,7,8-HxCDD	93	90	85	98	90	87	80
1,2,3,6,7,8-HxCDD	82	80	81	93	86	102	74
1,2,3,7,8,9-HxCDD	93	87	85	69	95	88	83
1,2,3,4,6,7,8-HpCDD	83	77	75	81	89	84	87
OCDD	92	79	76	87	90	95	81
2,3,7,8-TCDF	72	74	72	75	85	72	62
1,2,3,7,8-PeCDF	90	85	86	83	88	92	80
2,3,4,7,8-PeCDF	90	88	85	86	84	87	81
1,2,3,4,7,8-HxCDF	79	74	78	78	89	78	69
1,2,3,6,7,8-HxCDF	76	73	75	68	84	69	70
2,3,4,6,7,8-HxCDF	74	71	72	68	87	72	67
1,2,3,7,8,9-HxCDF	82	76	70	77	97	74	70
1,2,3,4,6,7,8-HpCDF	80	76	74	78	93	80	74
1,2,3,4,7,8,9-HpCDF	75	71	67	70	87	72	65
OCDF	92	79	76	87	92	96	81
PCB 77	82	76	82	82	81	88	78
PCB 81	87	79	90	88	86	94	84
PCB 126	78	73	73	71	75	74	67
PCB 169	98	93	88	89	86	101	85
PCB 105	73	77	70	81	71	75	69
PCB 114	79	74	79	89	78	79	76
PCB 118	77	72	72	87	74	74	72
PCB 123	80	75	80	91	80	80	75
PCB 156	71	85	74	72	75	57	62
PCB 157	74	74	71	74	76	59	58
PCB 167	112	100	71	72	71	51	96
PCB 189	86	91	88	88	89	71	76
PCB 28	76	70	104	88	107	78	60
PCB 52	70	73	101	109	109	48	68
PCB 101	73	78	84	104	79	69	74
PCB 153	69	109	72	80	72	29	80
PCB 138	107	76	84	74	71	52	98
PCB 180	64	94	66	79	65	47	71
PBDE 28	86	88	96	82	101	95	93
PBDE 47	103	85	102	86	107	100	98
PBDE 99	107	104	118	97	102	102	96
PBDE 153	82	91	77	79	95	106	94
PBDE 154	89	86	102	72	96	96	85
PBDE 183	79	95	115	100	106	110	101
PBDE 209	74	99	97	89	106	102	96

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