

## **AUTOMATION OF CHEMICAL ANALYSIS OF PCDD/FS, DIOXIN-LIKE PCBS, INDICATOR PCBS AND POLYBROMINATED DIPHENYL ETHERS IN FOOD AND FEED**

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### **Abstract**

Polychlorinated dibenzo-p-dioxin (PCDD/Fs) and polychlorinated biphenyls (PCBs), dioxin-like and indicator, in the past two decades often have been subject of incidents with food or feed (1, 2, 3). In general there is a request for increased sample throughput and short turn around times. By far reaching automation, sample capacity has been increased to 100 samples per week with a turn around time of 2 to 4 days. In combination with the bioassay DR CALUX this development has successfully been utilized during incidents. Furthermore, the method was extended to PBDEs.

### **Introduction**

The detection of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like chlorobiphenyls (non-ortho and mono-ortho substituted CBs) in biological and environmental samples has been subject of intensive research. Due to the toxicological behaviour, of the total 210 dioxin congeners only the 17 2,3,7,8-chlorine substituted compounds are of concern. In biological species these toxic compounds are accumulated in fat so the applied methods are mainly focused on the analysis of these toxic compounds. The concentration in biological samples is very low, in general in the low pg/g range expressed and in environmental samples somewhat higher, partly depending on the congener. Therefore highly sensitive and specific methods are required combining, after extraction, several clean-up procedures. Prior to extraction <sup>13</sup>C labelled dioxins (internal standards) are added to the samples for identification and quantification purposes. The initially used methods were set up in several steps. After extraction with Soxhlet the extract was purified by a combination of three semi automated systems. The first step, removal of fat, was performed with Gel permeation chromatography (GPC). After an additional clean up with activated Al<sub>2</sub>O<sub>3</sub> (step 2), separation between planar compounds (dioxins) and non-planar compounds e.g. chlorobiphenyls is carried out with a carbon column (step 3). The final extract is concentrated down to 10 µl and analysed with gas-chromatography-high-resolution-mass spectrometry (HRGC-MS), which is at present, the best-suited technique combining sufficient sensitivity and specificity. The mass spectrometric method to determine the tetra through octa dioxins is based on United States Environmental Protection Agency protocols. These protocols describe the basic tuning and calibration of the hardware as well as criteria for identification and quantification with isotope dilutions and procedures for quality assurance and quality control. Included in our analysis is a standard QA programme e.g. determination of recovery of internal standards, accuracy of spiked samples and blanks. To express the toxic potency of the mixture of dioxins, the toxic equivalency factor (TEF) approach is used (4).

The capacity of the initial method was 40 samples per week with a turn around time of one week. Especially during a crisis, results of the first set of samples are urgently required for decision making. In order to improve the method in terms of capacity and turn around time a process of far reaching automation was started. The initial method has been routinely used for more than 10 years for a.o monitoring programs, food and feed incidents and for human blood (5).

### **Materials and methods**

#### **ASE**

In principle all samples are extracted using an accelerated solvent extraction system (ASE 200 Dionex). Prior to extraction sixteen <sup>13</sup>C labelled dioxins, four <sup>13</sup>C labelled non-ortho PCBs, eight <sup>13</sup>C labelled mono-ortho PCBs, six <sup>13</sup>C labelled indicator-PCBs and <sup>13</sup>C-PBDE 209 and PCB 198 internal

standard are added to the samples. Biological samples, including fish, are extracted three times with hexane/acetone (7:3, v/v) at 100°C and 1500 PSI during 10 minutes. If necessary samples are first freeze-dried. Fat and oil samples are directly dissolved in hexane. For animal feed the extraction is performed with toluene/ethanol (9:1, v/v), three times at 100 °C and 1900 PSI during 20 minutes. Extracts are concentrated down to < 0.1 ml and after addition of the <sup>37</sup>Cl-2,3,7,8-TCDD (clean-up standard) extracts are made up with hexane to 25 ml.

### **Powerprep**

The initially developed purification method is replaced by a comprehensive automated system, the so called Power-Prep™ (Fluid Management Systems, Waltham, USA). Extracts are transferred to the Power-Prep system and purified on an acid silica column, a neutral silica column, a basic alumina column and an activated carbon/celite column. For the elution of the columns, custom made solvents and mixtures are used; hexane, hexane/dichloromethane (1:1, v/v) ethylacetate/toluene (1:1, v/v) and toluene (the program can be downloaded from the RIKILT website <http://www.rikilt.wur.nl/UK/services/Analyses/Dioxine+analysis/>).

The volume of the final extract is reduced to 0.5 ml using a turbovap. The recovery standards <sup>13</sup>C 1,2,3,4-TCDD and <sup>13</sup>C 2,3,4,6,7,8-HxCDF are added and the volume of the extract is again reduced to 0.5 ml using a turbovap.

### **GC-HRMS**

The HRGC/HRMS method was described previously (1). PCDD/F analysis was performed by GC-HRMS using an Agilent (Wilmington, USA) 6890 Series gas chromatograph and an AutoSpec Ultima high resolution mass spectrometer (Waters, Milford, USA). The GC column is a DB5 MS (60 m, 0.25 mm i.d., 0.25 µm; J&W, Folsom, USA). The mass spectrometer is operated in electron impact ionization mode, using selected-ion monitoring. Of the fraction containing the mono-ortho PCBs and the indicator PCBs a splitless injection of 2 µl is used to introduce the sample on the GC. After data reduction, results are directly transferred to a Laboratory Information Management System (LIMS) and after approval reported to the authorities.

### **LVI**

Injection of 100 µl of the extract containing the dioxins and non-ortho PCBs is carried out with a CIS-3 PTV injector (Gerstel, Germany) in solvent-vent mode with a vent flow of 100 ml/min. and a vent pressure of 100 Pa. The initial temperature of the PTV is 70 °C. Carbon dioxide is used for cooling the injector at this stage. After injection the temperature of the PTV is raised to 280 °C with 720 °C/min.

### **GC-MS NCI**

The fraction from the automated purification system which contains the mono-ortho and indicator PCB's also contains one group of flame-retardants, namely PBDEs (PBDE 17-28-47-66-71-85-99-100-138-153-154-183-190-209). A part of the fraction is after addition of the recovery standard (PCB 209) is injected into PTV-GC-MS. The GC is equipped with a 30 meter Cl-pesticide capillary column and ionization is done at 70eV via negative chemical ionization (NCI) using methane.

### **GCxGC-TOF-MS**

For GCxGC-TOF-MS analysis of the extracts, a Pegasus-4D system (LECO, St. Joseph, MI, USA) equipped with a CIS-4 PTV injector (Gerstel, Germany) was used. Comprehensive gas chromatography was facilitated by a two-stage modulator. Liquid nitrogen used for cold pulses was automatically filled into a Dewar using a liquid leveller, which accessed a 120 l liquid nitrogen storage tank. In the first dimension a 30m×0.25mm I.D., 0.25 µm RTX-CL pesticides column (Restek, Breda, The Netherlands) was used. The second dimension column was a 2m×0.1 mm, 0.1µm BPX-50 column (SGE, Darmstadt, Germany), mounted in a separate oven installed within the main GC oven. Helium was used as carrier gas at a constant pressure of 324 kPa. From the cleaned extract, 10 µl was injected into the PTV with a multi-baffled liner. The second-dimension separation time (modulation time) was 4.5 s. The data acquisition rate was 200 scans/s, covering a mass range of 50–1000 *m/z*.

## Results and discussion

Advantages of the new approach are obvious. The use of accelerated solvent extraction benefits in speed of extraction using less solvent and less bench space. Previously sample purification was time consuming. The first sample was ready for injection on the GC-HRMS after 18 hours. After that every fourth hour a new sample could be measured resulting in a maximum capacity of 40 samples per week with a turn around time of 18 hours for the first sample to more than 1 week for a series of maximally 40 samples. By using the automated system samples including procedure blanks and a reference sample, four real samples can be ready for analysis within four hours. By working in two shifts the capacity of the sample preparation can be increased to more than 100 real samples per week.

Tabel 1: Results of the validation

"analyt"	Average level (pg TEQ/g fat)	Average Accuracy %	Repeatability RSD %	Total reproducibility RSD %
PCDD/F-DL-PCB-TEQ	5.1	102	2.5	2.5
	7.3	98	1.7	1.7
	14.9	100	1.3	1.5
PCDD/F-TEQ	2.0	97	4.3	5.0
	2.9	93	3.1	3.4
	5.8	94	3.3	3.4
DL-PCB TEQ	3.1	105	1.8	3.2
	4.5	102	1.3	2.4
	9.2	104	1.2	2.3
MO-PCB-TEQ	0.97	101	1.4	1.8
	1.4	101	1.0	1.8
	3.0	104	0.9	1.4
NO-PCB-TEQ	2.1	106	2.5	4.3
	3.0	102	1.8	3.3
	6.2	104	1.5	3.1
SUM ind. PCBs	3100	102	2.8	3.6
	4500	100	2.3	3.7
	9300	104	1.5	2.6

Another time-consuming aspect of the previously used method is concentration to the final volume. In order to achieve the requested Limit of Detection (LOD) of 0.15 pg TEQ/gram fat for dioxins only, extracts had to be concentrated to a final volume of 10 µl. In the previous method this was done in several steps. The first step using the turbovap is still in use. After the turbovap the remaining extract of 0.5 ml was transferred to a calibrated glass tube of 10 ml. Final concentration at first to a volume of approximately 200 µl in the glass tube was done at 40°C under a gentle stream of nitrogen. The inner wall of the glass tube was rinsed with hexane and the extract was again concentrated now to a volume around 100 µl. After that this steps was once again repeated but now to the final volume of 10µl. This manual process is very critical and took at least 45 minutes. The risk of the described procedure is, due to unobservance for only a minute resulting in complete dryness of the extract, in loss of especially the more volatile PCBs and tetrachlorinated dioxins. To overcome this process, the GC-HRMS was equipped with a Large Volume Injector (LVI). After careful adjusting of the parameters and selection of the optimal glass liner we are now capable of routinely injecting 100 µl of the obtained extract without peak broadening. By injecting the sample in this way, it is no longer

obligatory to concentrate the sample to a final volume of 10 µl. In the new method, concentration is performed only with the turbovap to a final volume of 0.5 ml.

Overall capacity with the new method is approximately 100 samples per week with a turnaround time of 2-4 days. The bottleneck of the method is the GC-HRMS analysis. Runtime per analysis is due to the length of the capillary column 60 minutes. In the near future the column will be replaced by a column with a length of 30 m and an internal diameter of 0.15 µm. After data reduction, results are directly transferred to SQL LIMS resulting in faster reporting time and prevention of errors.

The newly adjusted method has been validated using 21 uncontaminated butterfat samples from Ireland. These samples have been analysed in three groups of seven samples on three different days. Samples were analysed as such and fortified at three different levels for PCDD/F-TEQ: 2/3 MRL, MRL and 2 MRL. The fortification with dioxin-like PCBs was at similar levels. The results of the validation study, accuracy, repeatability and reproducibility at the different levels of fortification are shown in table 1

Since recently, simultaneously with the analysis of the mono-ortho and indicator PCBs, the flame retardants are analyzed. This part of the method will be validated in due course. So far only fatty fish contains PBDEs above the LOD of 0.5 ppb for each congener.

Samples which show elevated levels of dioxins, PCBs or PBDEs are always analyzed using the GCxGC-TOF-MS. This technique, which is validated for the routine analysis for pesticides (6) is especially applied to get additional information about the presence of other compounds to be used for e.g. source identification.

In 2006 RIKILT was involved in a dioxin incident caused by recycled fat from gelatin production (1). Additional studies on the recycled fat with GC-TOF-MS revealed the presence of many contaminants including several PAHs and tri- and tetrachlorophenols at levels around 30 and 10 µg kg<sup>-1</sup>. In particular these types of chlorinated phenols are a well-known source of lower chlorinated dioxins.

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