

Automated rapid analysis for dioxins and PCBs in food, feedingstuff and environmental matrices

Kai Hölscher¹, Armin Maulshagen¹, Hamid Shir Khan², Guenter Lieck³, Peter A. Behnisch¹

¹eurofins-GfA, Münster

²Fluid Management Systems, Inc., Waltham

³University of Applied Science, Steinfurt

Introduction

Today there is a need to develop high throughput specific and sensitive methods for the determination of dioxins, dioxin-like PCBs and indicator-PCBs to ensure their rapid and reliable quantification in several kinds of food and feedingstuffs. Ideally one method would fit for several matrices with highest quality standards and with the possibility of a cost/time-effective samplehandling. However, generally in case of the numerous different PCDD/Fs, dioxin-like PCBs and indicator-PCBs as well as the large concentration range to cover this is quite difficult to fulfill. The implementation of an automated sample-treatment flow process (“dioxin street”), which contains an accelerated solvent extraction (ASE)¹⁻⁶, a Power-Prep workstation (Fluid Management Systems, FMS)⁷⁻¹² for automated clean-up, a Syncore Polyvap (Büchi, Switzerland) for solvent evaporation and a HRGC/HRMS (VG AutoSpec) analysis as detection method for several kinds of different matrices is described here. The aim of the present study is to confirm the high quality, low limits of quantification (LOQ), low PCB background levels¹³⁻¹⁶ and reliability of the Power-Prep system in combination with ASE extraction for dioxins, dioxin-like PCBs and indicator-PCBs.

Methods and Materials

All solvents were of high purity (Promochem). Different kinds of samples have been analysed coming from different locations in Europe (see Tables 1-3). Prior to extraction, the samples were fortified with ¹³C₁₂-labelled internal standards. Several different sample pre-treatments are described in Table 1. The ASE extractions were carried out on ASE 300 (Dionex, Sunnyvale, CA). All PCDD/F and PCB analyses were performed by HRGC/HRMS and quantification by means

of HP5890/VG AutoSpec systems. Three columns, DB-5MS (dioxins/non-ortho-PCBs), SP-2331 (dioxins) and HT5 (PCBs) were used for the chromatographic separation of the different PCDD/F and PCB congeners.

ASE Strategy:

ASE or Pressurised Liquid Extraction (PLE) is one of the most widely used techniques to replace the traditional soxhlet extraction¹⁻⁶. Different strategies have been employed depending on the different kinds of matrices. Usually the extraction temperature was between 100-185°C and the pressure was 1500 psi. Basically, samples have been prepared in 66 or 100 ml cells and statically extracted 2-3 times by different solvents (e.g. for milk: n-hexane/dichloromethane/methanol; for fatty food/feedingstuff: n-hexane/dichloromethane or in case of non-fatty food/feedingstuff or environmental samples: toluene), under the condition of a static time of 3-10 min, a flush volume of 80-125 % and a purge time of 90-120 seconds. In order to compare the efficiency of ASE to the soxhlet extraction, several samples have been extracted with toluene/acetone under a reflux condenser for 16 hours in a soxhlet apparatus.

Power-Prep workstation strategy:

Using this system it is possible to clean-up 6 samples in parallel simultaneously in less than 90 minutes (total cycle time between 2 runs of about 3 hrs). Moreover, high recoveries and an excellent precision for several target compounds in different matrices are achieved. The amount of co-extracted fat is usually around 0.5-10 g (max.) and a maximum of 4 g fat is in most cases diluted in 14 ml hexane and injected into the Power-Prep workstation. In case of some fat samples higher dilutions have been used to load on the Power-Prep system.

Results and Discussion

For end of 2004 the EU plans to include dioxin-like PCBs (12 WHO-PCBs) as regards the feed and food control. Therefore, reliable and sensitive methods are required for these PCB congeners for all kinds of different feed and food matrices. In our study we used an automated clean-up system called Power-Prep (FMS) for the sample clean-up and separation of dioxins and different PCB-fractions. Figure 1 shows the separation of dioxins/non-ortho-PCBs (4 coplanar WHO-PCBs) and mono-/di-ortho PCBs (8 non coplanar WHO-PCBs) by the Power-Prep system.

SAMPLING, CLEAN-UP AND SEPARATION

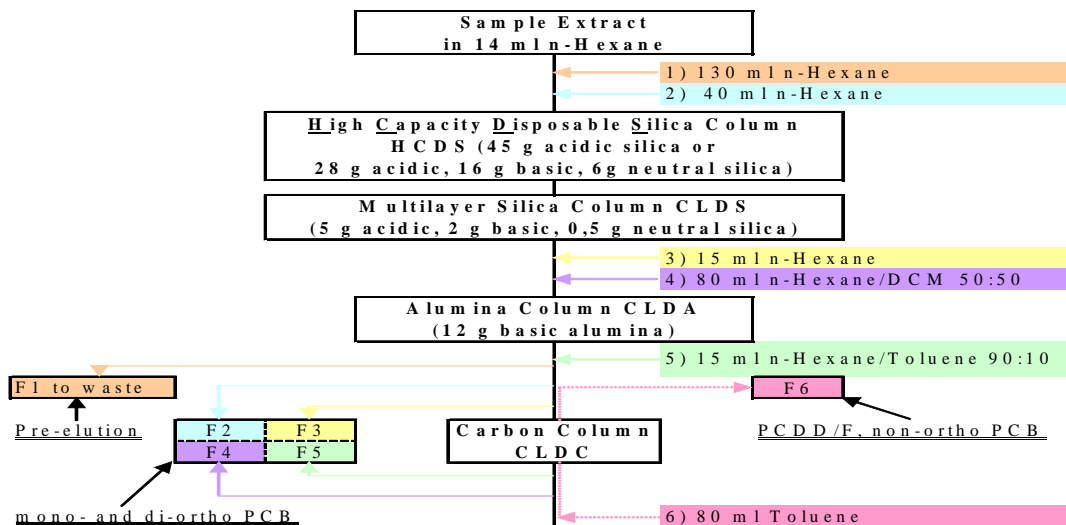


Figure 1: Flow chart explaining the separation of dioxins/non-ortho-PCBs (4 coplanar WHO-PCBs) and mono-/di-ortho PCBs (8 non-coplanar WHO-PCBs) by Power-Prep.

Recoveries for several kinds of matrices have shown to be in the range of 50-120 % (see Table 1). These findings are similar to the results reported by Pirard (2002)⁹. The limit of quantification using this automated dioxin/PCB clean-up system is lower than required by the EU guidelines (one fifth of the level of interest, see Table 2).

Table 1: Sample description with pre-treatment methods, sample amounts, choice of Power-Prep workstation columns and recoveries for PCDD/Fs and PCBs. Additional typical ratios of dioxin-TEQs to PCB-TEQs for these tested samples are listed.

	Extraction	Weight	HCDS ^a	Recovery in %		Ratio Dioxin/ PCB-TEQ
				PCDD/ F	PCB	
Feed						
Soya	SOX	3.5 g fat	X	90-101	56-100	0.04/0.11
Fish meal	ASE	3.5 g fat	X	80-110	70-100	0.49/1.1
Citrus pellets	ASE	20.0 g d.m.	X	59-120	71-107	0.04/0.08
Magnesium-sulphate	HCl & liq./liq.	30.0 g d.m.	-	74-107	72-100	0.07/0.06
Food						
Borecole	SOX	5.0 g d.m.	X	77-120	76-107	0.82/0.11
Olive oil	-	3.5 g	X	79-112	70-100	0.17/0.17
Meat	ASE	3.5 g fat	X	62-109	80-113	0.38/0.20
Baby food	ASE	3.5 g fat	X	83-108	-	-
Salmon	ASE	3.5 g fat	X	75-104	82-109	2.4/6.7
Milk	ASE	3.5 g fat	X	67-105	65-100	0.5/1.2
Environment						
Effluents	liq./liq. & SOX	3.0 l	-	76-110	50-100	1.7/0.5
Fly ash	HCl, liq./liq. & SOX	5.0 g d.m.	-	94-117	75-100	0.01/0.0008
Stack gas	SOX	0.5 m ³	-	87-120	63-101	3.1/0.78
Ambient air	SOX	20 m ³	-	86-120	68-100	17/0.27
Soil	SOX	2.5 g d.m.	-	76-112	68-105	0.81/0.50
Dust fall	SOX	0.5 m ² *d	-	82-115	69-100	0.003/0.003

^aHigh-capacity disposable silica column before the CLDS, CLDA and CLDC Power-Prep column

Table 2: Limit of Quantification (LOQ) for different matrices required by the EU legislatives 2002/69 and 2002/70 analysed by ASE, Power-Prep and HRGC/HRMS (pg WHO-TEQ/g).

Matrix	Action level	LOQ PCDD/Fs	LOQ PCBs
Feedingstuff	0.75 ^a	0.02	0.01
Minerals	1.0 ^a	0.02	0.01
Animal fat, incl. milk fat and egg fat	2.0 ^a	0.2	0.1
Fish oil	6.0 ^a	0.2	0.1
Fish	1.25 ^a	0.2	0.1
Pig meat	1.0 ^b	0.2	0.1
Muscle meat fish	4.0 ^b	0.3	0.2
Milk, milk products	3.0 ^b	0.2	0.1
Eggs/egg products	3.0 ^b	0.2	0.1
Vegetable fat	0.75 ^b	0.2	0.1

^aFeedingstuffs (ng WHO-TEQ/kg d.m.); ^bFood (pg WHO-TEQ/g fat)

Table 3 shows typical congener-specific recoveries for dioxins, dioxin-like PCBs and indicator-PCBs.

Table 3: Congener-specific typical recoveries for PCDD/Fs and PCBs for different matrices.

Congeners	Feed	Milk	Fish	Meat	Oils/ fats	Vege- tables	Feed addi- tive	Mean
PCDD/Fs								
T4CDD	98	105	87	108	82	81	97	90
P5CDD	88	93	85	86	98	101	97	93
H6CDD	85	103	83	94	91	97	93	91
H7CDD	94	94	81	92	78	84	94	87
O8CDD	105	99	92	100	86	83	96	93
T4CDF	93	104	76	103	88	85	98	90
P5CDF	73	83	73	82	96	98	92	87
H6CDF	78	88	80	88	96	80	86	85
H7CDF	109	95	86	101	77	83	99	91
O8CDF	108	97	92	100	91	80	95	91
Indicator- PCBs								
T3CB-28	90	91	82	70	97	87	80	82
T4CB-52	85	91	81	67	79	76	78	78
P5CB-101	95	81	82	60	105	84	90	85
H6CB-153	92	86	97	71	94	93	78	86
H6CB-138	98	99	94	92	96	87	100	93
H7CB-180	100	93	107	92	100	98	88	96
WHO-PCBs								
T4CB-81	77	91	82	94	93	88	72	84
T4CB-77	84	94	85	98	96	90	75	88
P5CB-123	85	72	95	95	93	104	97	90
P5CB-118	85	81	82	97	92	90	89	87
P5CB-114	90	76	81	105	104	93	95	90
P5CB-105	84	77	89	86	100	108	84	87
P5CB-126	116	119	105	119	114	112	112	112
H6CB-167	84	83	97	75	96	96	92	88
H6CB-156	81	82	105	90	100	91	92	90
H6CB-157	76	88	105	93	85	91	93	89
H6CB-169	109	89	103	84	111	116	103	106
H7CB-189	77	82	108	76	93	79	85	85

Several publications already described the difficulties of dioxin-like PCB congeners in the clean-up and HRGC/HRMS measurement. Especially, laboratory-background levels of the PCB-congeners PCB-118, PCB-156 and PCB-105 have been reported to be a critical issue for ultra-trace analyses of dioxin-like PCBs¹³⁻¹⁵. Therefore, we studied the differences of PCB background levels using a combination of classic acid silica, alumina and carbon glass columns in comparison to the same combination of usual Power-Prep columns and special designed PCB-free Power-Prep columns (incl. HCDS). Especially, the levels for the PCBs in higher concentrations in blank samples such as the indicator-PCBs and PCB-105, PCB-118, PCB-156 or PCB-167 could be significantly reduced (Figure 2).

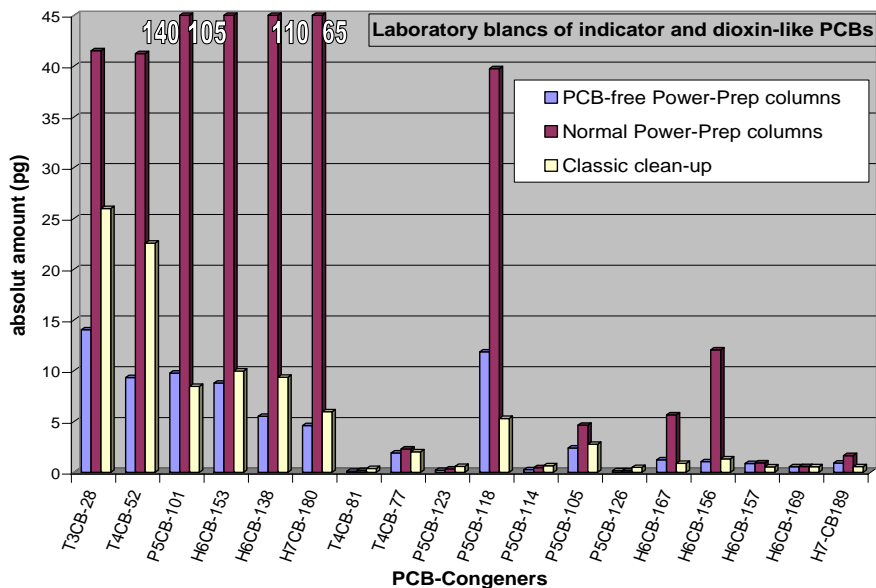


Figure 2: Laboratory blank levels of indicator and dioxin-like PCBs with PCB-free (n=4) and normal (n=3) Power-Prep columns compared to classic clean-up columns (n = 6 per clean-up)

Figure 3 shows the better ability to separate coplanar PCBs (e.g. PCB-77/PCB-81) from background interferences by using the silica gel/sulphuric acid, alumina oxide and carbon columns with our separation method compared to the classic clean-up method using the same combination of clean-up columns.

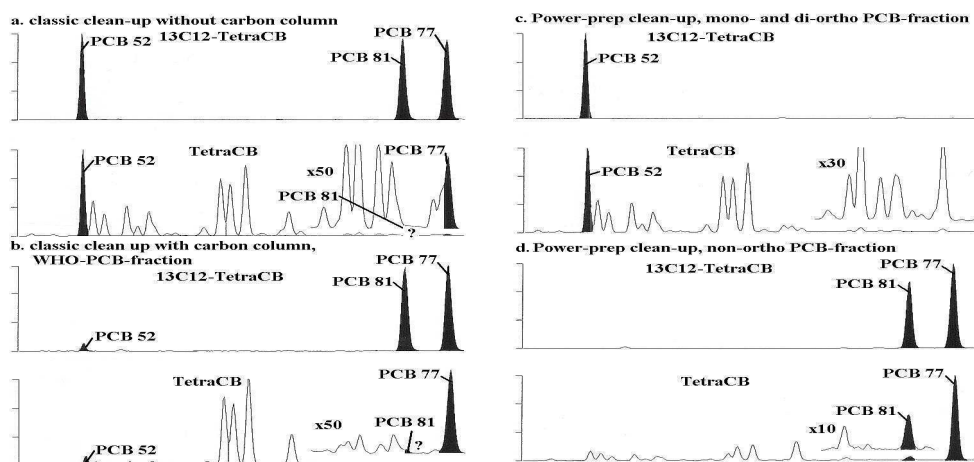


Figure 3: Comparison of the separation of several T4CB congeners by classic clean-up (with and without carbon column) and by using the Power-Prep method.

Conclusions

On-line automated extraction and clean-up of several kinds of feed, food and environmental samples using a combination of ASE, Power-Prep workstation, Synchro Polyvap solvent evaporation and finally HRGC/HRMS analysis has been critically tested.

The Power-Prep method in combination with ASE extraction developed and tested by us showed high recovery rates, low laboratory-background levels and no chromatographic interferences for the coplanar PCBs and, therefore, fulfills all requirements for the standard dioxin, dioxin-like PCB and indicator-PCB analyses.

The automated “dioxin street” considerably simplifies the sample handling and thus, reduces possible sources of errors. It decreases the turn-around time by a factor of 2-3 times as well as it increases the precision and accuracy of the analysis. The amount of repeated analyses could be significantly reduced and the GC capillary column resists longer.

This system has been proved to be very flexible and reliable for several kinds of feed, food and environmental samples. The new PCB-free columns from FMS lead to lower PCB background levels, LOQ levels and, therefore, lower upperbound concentrations (or the possibility to use a lower amount of sample).

We, therefore, can confirm a significant reduction of costs and time by applying such automated extraction and clean-up systems¹⁸.

References

1. Sorring S., Wiberg K., Björklund E. and Haglund P. (2003) *Organohalogen Compounds* 60, 1.
2. Hosseinpour J., Rottler H. and Malisch R. (2003) *Organohalogen Compounds* 60, 13.
3. Misita M., Schrock M., Tracy K. and Tabor J (2003) *Organohalogen Compounds* 60, 37.
4. Sorring S. and Björklund E. (2003) *Organohalogen Compounds* 60, 53.
5. Grochawalski A. and Maslanka A. (2003) *Organohalogen Compounds* 60, 65.
6. Archer J.C., Mobley R.J., Shojaee S., Pence L.M. and Earnheart C. *Organohalogen Compounds* 60, 207.
7. Matsukami H., Hayashi A., Takasuga T., Ohi E. and Senthilkumar K. (2003) *Organohalogen Compounds* 60, 73.
8. Pirard C., de Pauw E. and Focant J.-F. (2003) *J. of Chromatography* 998, 169.
9. Pirard C., Focant J.-F., and de Pauw E. (2002) *Anal. Bioanal. Chem.* 372, 373.
10. Llerena J.J., Abad E., Caixach J. and Rivera J. (2003) *Chemosphere* 53, 679.
11. Focant J.-F., Eppe G., Pirard C., Massart A.-C., Andre J.-E. and de Pauw E. (2002) *Chemosphere* 48, 167.
12. Focant J.-F., Pirard C., Massart A.-C. and de Pauw E. (2003) *Chemosphere* 52, 725.
13. Datta S., Mower B. And Damaske E. T. (2003) *Organohalogen Compounds* 60, 371.
14. Ferrario J., Byrne C. and Dupuy A. E. (1997). *Chemosphere* 34, 2451.
15. She J, Petreas M.X., Visita P., Mc Kinney M., Sy F.J., Winkler J.J., Hooper K. and Stephens R.D. (1998) *Chemosphere* 37, 431.
16. Behnisch P. (1997) *Nicht-, mono- und di-ortho-chlorierte PCBs. Band 321*, UFO Verlag, Konstanz, ISBN 3-930803-2-8.
17. Focant J., Pirard C., Eppe G., Shirkhan H. and de Pauw, E. (2003) *Organohalogen Compounds* 60, 421.
18. Webb, L. (2003) *Organohalogen Compounds* 60, 484.