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PCDD/PCDF DETERMINATION IN FEEDING STUFFS BY MEANS OF ACCELERATED SOLVENT EXTRACTION (ASE) AND HRGC/HRMS

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Introduction

Today there is a major concern regarding the quality of food and feeding stuffs because of the feed poisoning episode that occurred in Belgium in May 1999 [1]. Various feeding stuffs then contained high levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/PCDF) [2]. This contamination caused extraordinary high levels of PCBs and PCDD/PCDF in a number of meat, eggs and other animal food samples. In order to reduce the contaminant accumulation in animal food, their occurrence in feeding stuffs should be minimized. Numerous analyses of various feeding stuffs are mandatory to characterize the ubiquitous background exposure and to trace back the sources of contamination as soon as they are identified. However, determination of PCDD/PCDF at trace levels is a challenge that requires complicated and very time-consuming sample extraction and clean up procedures. In order to drastically minimize the analysis time, we checked the potential of an accelerated solvent extractor (ASE) for merely extraction and an ASE method with integrated sulphuric acid clean-up as a substitute for the standard Soxhlet extraction method.

Materials and Methods

Reagents:

- Native and ^{13}C-labelled PCDD/PCDF standards were purchased from Promochem, Germany
- Solvents used were of quality “Nanograde” and purchased from Promochem, Germany
- A feeding stuff which serves as a quality control pool in our laboratory since a couple of years was used as the reference sample

Apparatus:

- ASE: Dionex ASE 300, cell size 100 ml
- GC/MS: Agilent HP 6890/Micromass AutoSpec Ultima HRMS

Extraction procedures:

a) Soxhlet extraction:

15 g feeding stuff were mixed with 60 g sodium sulphate, placed into a glass fiber cartridge and fortified with internal standards. The extraction took place in a Soxhlet extractor with toluene/ acetone 70/30 for 16 hours overnight

b) accelerated solvent extractor (ASE):

15 g feeding stuff was mixed with 10 g glass granulate and filled into the extraction cell together with glass granulate/diatomeous earth as shown in Fig. 1.

Extracts a and b were cleaned up after the evaporation of the solvents on a sulphuric acid column.

c) accelerated solvent extractor (ASE) with integrated sulphuric acid clean-up

The extraction cell was filled with a mixture of 15 g sample and 10 g glass granulate as well as silica gel coated with sulphuric acid as depicted in Fig. 2.

Extractions b and c both were performed with cyclohexane using the conditions shown in Table 1.

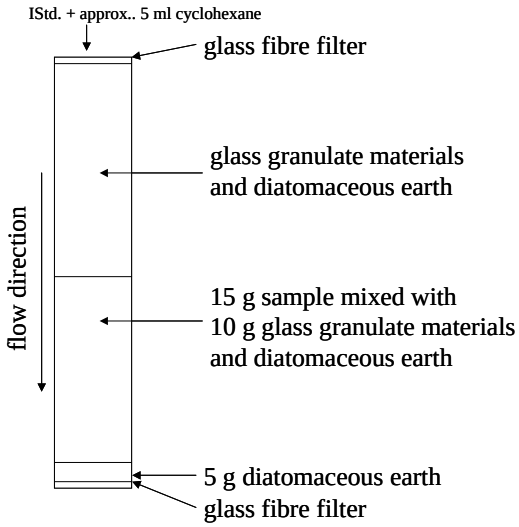

Figure 1: Packing of the ASE extraction cell

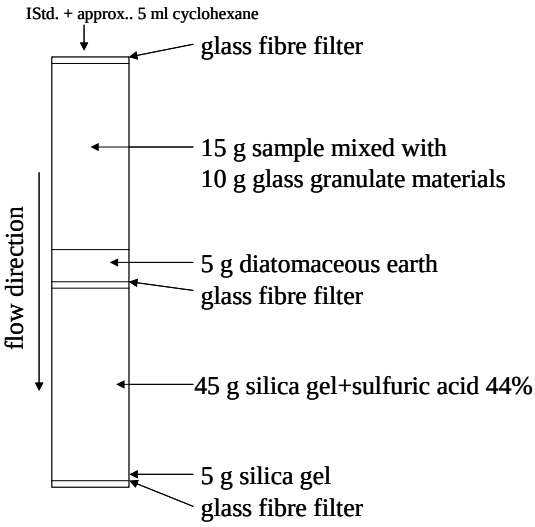

Figure 2: Packing of the ASE extraction cell with integrated sulphuric acid clean-up

Table 1: ASE conditions

Standard extraction parameters used in all ASE experiments unless otherwise stated			
Temperature	120°C	Cycle	4
Static time	10 min.	Pressure	1500 psi
Heat time	5 min.	Flush volume	80 %
Purge time	100 sec.	Cell volume	100 ml

Clean up with florisil:
After evaporation of the solvents, the extracts a – c were dissolved in 0.5 ml toluene and applied onto a chromatography column filled with 6 g florisil in n-hexane and a thin layer of sodium sulphate on the top. The first eluate of 80 ml n-hexane contains inter alia PCBs and was discarded for this investigation. The PCDD/PCDF elution was performed with 120 ml toluene.
Clean up with active carbon:
For the packing of four chromatography columns 0,18 g carbopack C and 0,82 g celite 545 were mixed thoroughly and filled into chromatography columns (ID: 8 mm; length: 100 mm). The column was rinsed with 15 ml toluene, 5 ml dichloromethane/methanol/toluene (75/20/5), 5 ml dichloromethane/cyclohexane (1/1) and 10 ml n-hexane before the residue of the clean up with florisil, dissolved in 1 ml n-hexane, was applied onto the column. The first effluent, 2 ml n-hexane and 1 ml dichloromethane/methanol/toluene (75/20/5) was discarded. The PCDD/PCDF elution was performed with 30 ml toluene. After addition of a further syringe spike, the extract was evaporated in a gentle stream of nitrogen, reconstituted with 12 µl toluene and transferred into an auto sampler vial for HRGC/HRMS analysis.

GC/MS:
Agilent 6890/Micromass Autospec Ultima HRMS
Injector: 275°C; Column: DB-dioxin (J&W) 60 m, 0,15 µm film thickness, 0,25 mm ID;
Temperature programme: 75°C (3 min) - 195°C (15°C/min) - 270°C (3°C/min)
Carrier gas: helium, pressure: 2 bar; MS-Resolution: 10000

Results and Discussion
Table 2 summarizes the recoveries of the isotope labelled PCDD/PCDF from the feeding stuff samples after the different extraction methods. The percentage recovery data are important parameters to compare different methods, mainly in extraction procedures. In all extraction experiments the average recoveries of dioxins were good (63-102%) and they were comparable.

Table 2: Recovery of isotope labelled PCDD/PCDF from feeding stuffs after different extraction procedures

Method	*Soxhlet*		*ASE*		*ASE with sulphuric acid*	
^{13}C-labelled standard solution	Sample	Blank	Sample	Blank	Sample	Blank
polychlorinated dibenzo-p-dioxins						
2,3,7,8-TCDD	87	85	94	102	102	96
1,2,3,7,8-/1,2,4,6,7-/1,2,4,8,9-PCDD	95	92	90	95	92	86
1,2,3,4,7,8-HxCDD	93	88	88	80	89	83
1,2,3,6,7,8-HxCDD	92	90	83	89	88	83
1,2,3,7,8,9-HxCDD	92	88	84	87	87	83
1,2,3,4,6,7,8-HpCDD	82	87	79	80	79	77
1,2,3,4,6,7,8,9-OCDD	71	73	72	65	70	72
polychlorinated dibenzofurans						
2,3,7,8-TCDF	79	83	67	81	73	69
1,2,3,7,8-PCDF	97	94	97	98	98	93
2,3,4,7,8-PCDF	89	87	91	90	88	86
1,2,3,4,7,8-/1,2,4,6,8,9-HxCDF	89	88	85	81	89	83
1,2,3,6,7,8-HxCDF	91	89	88	90	92	86
2,3,4,6,7,8-HxCDF	88	85	79	84	80	77
1,2,3,7,8,9-HxCDF	80	76	73	72	79	69
1,2,3,4,6,7,8-HpCDF	71	77	90	72	73	90
1,2,3,4,7,8,9-HpCDF	76	81	72	74	76	72
1,2,3,4,6,7,8,9-OCDF	63	74	69	66	65	71

Additional evaluation of the three extraction methods were obtained by analysis of the feeding stuff control pool. Table 3 shows the dioxin levels of the sample obtained with the three different methods. All values obtained were within uncertainty limits and close to the values of the Soxhlet extraction. Furthermore, the SDs of the measured values were small. These results indicate that the present methods are reliable for the analysis of dioxins in feeding stuffs. The conditions presented in Table 1 may be further adjusted to optimise the extraction efficiency. Obviously, this optimisation depends on the matrix. Particularly, the ASE with integrated sulphuric acid clean-up depends

on the fat content of the feeding stuff samples. The weight of the feeding stuff has to be adapted at a fat content of more than 2,2 g (15 %).

Table 3: Comparison of dioxin levels in the feeding stuff pool between three analytical methods

Method	*Soxhlet*			*ASE*			*ASE with sulphuric acid*		
	ng/g	SD	VK %	ng/g	SD	VK %	ng/g	SD	VK %
TEQ (WHO, 1997)	0,959	0,095	9,9	0,802	0,045	5,4	0,814	0,025	3,0
TEQ (WHO, ½ LOD)	0,960	0,095	9,9	0,802	0,045	5,4	0,815	0,025	3,0
polychlorinated dibenzo-p-dioxins									
2,3,7,8-TCDD	0,157	0,016	10,4	0,120	0,014	9,4	0,130	0,014	10,9
1,2,3,7,8-/1,2,4,6,7-/1,2,4,8,9-PCDD	0,213	0,023	10,6	0,190	0,000	0,0	0,200	0,000	0,0
1,2,3,4,7,8-HxCDD	0,054	0,008	15,6	0,050	0,007	20,2	0,040	0,000	0,0
1,2,3,6,7,8-HxCDD	0,154	0,016	10,7	0,130	0,021	15,7	0,130	0,000	0,0
1,2,3,7,8,9-HxCDD	0,064	0,011	16,8	0,055	0,007	10,9	0,045	0,007	15,7
1,2,3,4,6,7,8-HpCDD	2,470	0,231	9,4	1,800	0,071	3,6	1,850	0,071	3,8
1,2,3,4,6,7,8,9-OCDD	17,41	2,619	15,0	12,10	0,778	6,2	12,55	0,212	1,7
polychlorinated dibenzofurans									
2,3,7,8-TCDF	0,929	0,106	11,4	0,950	0,057	6,2	0,835	0,035	4,2
1,2,3,7,8-PCDF	0,209	0,023	10,9	0,175	0,007	3,8	0,170	0,000	0,0
2,3,4,7,8-PCDF	0,657	0,077	11,7	0,575	0,042	7,1	0,585	0,007	1,2
1,2,3,4,7,8-/1,2,4,6,8,9-HxCDF	0,472	0,200	42,4	0,150	0,007	4,6	0,145	0,007	4,9
1,2,3,6,7,8-HxCDF	0,130	0,012	9,6	0,100	0,007	7,4	0,100	0,000	0,0
2,3,4,6,7,8-HxCDF	0,226	0,019	8,4	0,195	0,007	3,4	0,195	0,007	3,6
1,2,3,7,8,9-HxCDF	-0,003	n.d.	n.d.	-0,010	n.d.	n.d.	-0,020	n.d.	n.d.
1,2,3,4,6,7,8-HpCDF	1,720	0,235	13,6	1,100	0,000	0,0	1,150	0,071	6,1
1,2,3,4,7,8,9-HpCDF	0,189	0,039	20,5	0,120	0,042	28,3	0,120	0,000	0,0
1,2,3,4,6,7,8,9-OCDF	15,29	1,535	10,0	10,00	0,919	8,5	10,95	0,636	5,8
Sum PCDD and PCDF	48,85	5,227	10,7	27,81	1,874	6,4	22,08	0,643	2,9

The data presented show that ASE is essentially equivalent to conventional extraction techniques for dioxin analyses in feeding stuff samples. Moreover, ASE with integrated sulphuric acid clean-up is a promising analytical tool that does not only drastically reduce the amount of solvent but also reduces working and analysis time because extraction and primary clean-up are automatically performed in one step within 60 minutes.

References

1. European Commission (1999) Offic. J. Eur. Commission L310. 62
2. Bernard A., Hermans C., Broeckaert F., De Poorter G., De Cock A., Housins G. (1999) Nature 401, 231