COMPARISON OF ACCELEERATED SOLVENT EXTRACTION (ASE) WITH INTEGRATED SULPHURIC ACID CLEAN UP AND SOXHLET EXTRACTION FOR DETERMINATION OF PCDD/PCDF, DIOXIN-LIKE PCB AND INDICATOR PCB IN FEEDING STUFFS

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Introduction

Currently there is increasing public awareness of food and feeding stuff quality. The crisis in Belgium once again put focus on polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/PCDF) and polychlorinated biphenyls (PCB) as a result of contamination of feeding stuffs[1]. In order to handle such crises, rapid and reliable methods must be available to instantly give relevant information for justifying administrative action. The determination of PCDD/PCDF and PCB at trace levels is a challenge that requires complicated and very time-consuming sample extraction and clean up procedures. The most frequently methods used for the determination of PCDD/PCDF and PCB in feeding stuffs combines Soxhlet extraction with clean up steps using different column chromatographies, such as silica gel coated with sulphuric acid, florisil, alumina and actived carbon. The final confirmative analysis is generally performed with capillary gas chromatography-high resolution mass spectrometry. This paper describes a faster and simple liquid extraction method for the determination of PCDD/PCDF, dioxin-like PCB and indicator PCB in feeding stuffs matrices and compares its results with those of a classical soxhlet method.

Methods and Materials

Reagents:

- Native and ^{13}C-labelled PCDD/PCDF, dioxin-like PCB and indicator PCB standards were purchased from Promochem, Germany
- Solvents used were of quality grade "Nanograde" and purchased from Promochem, Germany

Apparatus:

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

Extraction procedures:

a) Soxhlet extraction:

15 g feeding stuff is mixed with 60 g sodium sulphate, placed into a glass fiber cartridge and fortified with internal standards. The extraction takes place in a Soxhlet extractor with toluene/

acetone 70/30 for 16 hours overnight. The extract is cleaned up after the evaporation of the solvents on a silica gel column coated with sulphuric acid.
b) Accelerated solvent extraction (ASE) with integrated sulphuric acid clean-up:
The extraction cell is filled with silica gel coated with sulphuric acid as fat retainer, diatomeous earth as drying agent and a mixture of 15 g sample and 10 g silica gel with 44% sulphuric acid as depicted in Fig. 1. The extraction is performed with cyclohexane using the conditions shown in Table 1.
Clean up with florisil:
After evaporation of the solvents, the Soxhlet extract after the sulphuric acid clean up and the ASE extract are 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 top. The first eluate of 80 ml n-hexane contains inter alia PCBs (I). The PCDD/PCDF elution is performed with 120 ml toluene (II).

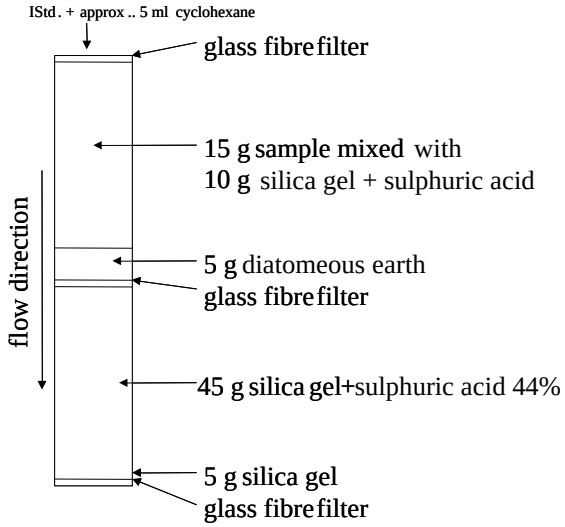

Figure 1: Packing of the ASE extraction cell with integrated sulphuric acid clean-up

Standard extraction parameters used in all ASE experiments unless otherwise stated

Temperature:	120°C
Static time:	10 min.
Heat time:	5 min.
Purge time:	100 sec.
Cycle:	5
Pressure:	1500 psi
Flush volume:	80 %
Cell volume:	100 ml

Table 1: ASE conditions

Clean up of the n-hexane fraction (I) with active carbon for dioxin-like PCB and indicator PCB:
5 g Chromosorb WHP (100/120 mesh) and 0,5 g activated charcoal SP-1 are mixed thoroughly and 0,1 g of this mixture is filled into chromatography columns (ID: 8 mm; length: 100 mm). The column is rinsed with 50 ml toluene and 50 ml dichloromethane/cyclohexane (1/1). The n-hexane residue (I) is dissolved in 0,5 ml n-hexane and applied onto the column. The first eluate of 200 ml dichloromethane/cyclohexane (1/1) contains the mono ortho PCB and indicator PCB. The extract is concentrated to 3 ml and after addition of a syringe spike and 10 µl dodecane, evaporated in a gentle stream of nitrogen and finally reconstituted with 15 µl toluene. The final solution of 25 µl (15µ toluene and 10 µl dodecane) is used for HRGC/HRMS analysis of mono-ortho and indicator PCB. The non ortho PCB elution is performed with 100 ml toluene. After addition of a syringe spike, the extract is evaporated in a gentle stream of nitrogen, reconstituted with 40 µl toluene and analysed also with HRGC/HRMS.

Clean up of the toluene fraction (II) with active carbon for PCDD/PCDF:
0,18 g Carbopack C and 0,82 g Celite 545 are mixed thoroughly and 0,25 g are filled into chromatography columns (ID: 8 mm; length: 100 mm). The columns are conditioned with 15 ml toluene, 5 ml dichloromethane/methanol/toluene (75/20/5), 5 ml dichloromethane/cyclohexane (1/1) and 10 ml n-hexane. The toluene residue (II) is dissolved in 1 ml n-hexane and applied onto the column. The column is rinsed with 2 ml n-hexane and 1 ml dichloromethane/methanol/toluene (75/20/5). The PCDD/PCDF elution is performed with 30 ml toluene. After addition of a syringe spike, the extract is 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 Analysis:
HRGC/HRMS: Agilent 6890/Micromass Autospec Ultima HRMS
PCB: Injector: 275°C; Column: DB-dioxin (J&W) 30 m, 0,25 µm film thickness, 0,25 mm ID; Temperature programme: 80°C (3 min) - 175°C (30°C/min) - 270°C (3°C/min)
PCDD/PCDF: Injector: 280°C; Column: DB-5MS (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

Method/ Concentration in pg/g product	ASE/H_2SO_4			Soxhlet		
	Average	SD	% RSD	Average	SD	% RSD
polychlorinated dibenzo-p-dioxins						
2,3,7,8-TCDD	0,12	0,01	5	0,12	0,01	5
1,2,3,7,8-PCDD	0,12	0,00	3	0,13	0,01	9
1,2,3,4,7,8-HCDD	0,10	0,01	9	0,11	0,02	14
1,2,3,6,7,8-HCDD	0,11	0,01	12	0,12	0,02	14
1,2,3,7,8,9-HCDD	0,13	0,01	7	0,13	0,01	11
1,2,3,4,6,7,8-HCDD	0,23	0,03	12	0,26	0,04	14
1,2,3,4,6,7,8,9-OCDD	2,31	0,10	4	2,54	0,24	9
polychlorinated dibenzofurans						
2,3,7,8-TCDF	0,45	0,03	7	0,48	0,04	8
1,2,3,7,8-PCDF	0,15	0,01	6	0,14	0,01	8
2,3,4,7,8-PCDF	0,23	0,01	5	0,24	0,01	5
1,2,3,4,7,8-HCDF	0,11	0,01	7	0,12	0,01	10
1,2,3,6,7,8-HCDF	0,10	0,01	5	0,10	0,01	10
1,2,3,7,8,9-HCDF	0,10	0,02	18	0,09	0,01	6
2,3,4,6,7,8-HCDF	0,10	0,02	17	0,11	0,01	10
1,2,3,4,6,7,8-HCDF	0,15	0,01	6	0,15	0,02	11
1,2,3,4,7,8,9-HCDF	0,12	0,02	13	0,11	0,01	10
1,2,3,4,6,7,8,9-OCDF	0,20	0,03	13	0,19	0,03	16
total WHO-TEQ (PCDD/F)	**0,48**	**0,01**	**2**	**0,50**	**0,03**	**5**

Table 2: Comparison of the classic Soxhlet extraction and the ASE method as procedures for the analysis of PCDD/PCDF in feeding stuff

In a previous publication[2] the recoveries of the isotope labelled PCDD/PCDF from the feeding stuff samples after the different extraction methods were summarized. In all extraction experiments the average recoveries of dioxins ranged between 63-102%. Table 2 shows the dioxin levels of a feeding stuff reference material obtained with the two different methods. All values obtained are

within uncertainty limits and the total WHO-TEQ (PCDD/F) are with 0,48 pg/g for the ASE method (2% RSD and 0,50 pg/g for the Soxhlet extraction (5% RSD) comparable. The results and relative standard deviations obtained for the analysis of dioxin like PCB and the indicator PCB are shown in table 3. The total WHO-TEQ (DL-PCB) and total WHO-TEQ from the ASE methods with 0,75 and 1,23 pg/g were also very close to the values of the Soxhlet extraction (0,83 and 1,33 pg/g). These results indicate that the present sample extraction and online clean up procedure are effective for the analysis of dioxins, dioxin-like PCB and indicator PCB in feeding stuffs.

Method / Concentration in pg/g product	ASE/H_2SO_4			Soxhlet		
	Average	SD	% RSD	Average	SD	% RSD
non ortho PCB						
PCB 77	16,6	1,8	10,8	19,6	1,5	7,6
PCB 81	1,6	0,2	13,8	1,9	0,2	11,6
PCB 126	4,5	0,3	5,9	5,4	0,6	10,5
PCB 169	1,6	0,1	3,5	1,9	0,3	13,4
mono ortho PCB						
PCB 105	305,0	30,3	9,9	315,8	27,5	8,7
PCB 114	21,0	1,4	6,7	23,7	2,7	11,5
PCB 118	1032,2	68,3	6,6	1103,2	77,7	7,0
PCB 123	98,8	11,0	11,1	118,7	20,7	17,4
PCB 156	153,0	12,3	8,1	168,3	14,7	8,8
PCB 157	23,3	1,2	5,2	24,3	1,6	6,7
PCB 167	122,8	10,7	8,7	137,2	12,7	9,3
PCB 189	13,0	1,4	10,9	15,3	1,0	6,7
total WHO-TEQ (DL-PCB)	**0,75**	**0,10**	**13,3**	**0,83**	**0,07**	**8,7**
total WHO-TEQ	**1,23**	**0,10**	**8,3**	**1,33**	**0,08**	**6,2**
Indicator PCB						
PCB 28	284,5	18,5	6,5	615,3	113,1	18,4
PCB 52	373,0	22,5	6,0	520,3	49,6	9,5
PCB 101	651,8	35,4	5,4	795,0	91,2	11,5
PCB 138	790,0	54,2	6,9	924,0	72,3	7,8
PCB 153	1151,7	104,1	9,0	1390,8	78,8	5,7
PCB 180	278,2	16,8	6,0	344,7	25,7	7,5

Table 3: Comparison of the classical Soxhlet extraction and the ASE method as a procedure for the analysis of PCB in a feeding stuff and to get the total WHO-TEQ

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 composition of the feeding stuff. This method isn't usable for roughage, like grass and lucerne flour, because of the higher amount of waxes. Also by a higher amount of choline chloride or sodium chloride the ASE extraction with integrated sulphuric acid clean up doesn’t work properly. Another important factor is the fat content. The weight of the feeding stuff has to be adapted at a fat content of not more than 5% because of the lower fat retainer capacity of the silica gel coated with sulphuric acid under the ASE condition with the high pressure and temperature.

The data presented show that ASE with integrated sulphuric acid clean up is essentially equivalent to conventional “classical” extraction techniques for dioxin and PCB analyses in feeding stuff samples. Moreover, the presented ASE method 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 90 minutes.

References

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2 Bernsmann, T.; Fürst, P. Organohalogen Compounds (2003) 60, 408-411