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DETERMINATION OF DIOXIN/PCB AND BDE IN ONE AUTOMATIC SYSTEM WITH DIFFERENT SAMPLE CLEAN-UP COLUMN SETTINGS

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Introduction

The recent years in POPs analysis have illustrated the need for fast and high throughput methods to identify and confirm non-compliant samples in the feed and food chain. The clean-up in many laboratories is still done manually. The clean-up from fat extraction until the final solution can take up to several days. The dioxin analysis requires a multi column clean-up. The mostly used columns are a sulphuric acid silica gel column followed by a Florisil or an alumina column and finally carbon column chromatography purification. Depending on the column settings different fractionation results can also be obtained. For this purpose, a highly efficient clean-up procedure is required to purify raw extracts prior to the final analytical separation and quantification step. So there is a need of automation to get purified extracts within 60 minutes. Since 2013 several new automatic sample clean up systems came into the market. Each system follows a single and fix column set-up that cannot be changed. But sometimes, depending on the matrix, it is necessary to change the column settings, to avoid interfering matrix effects. To offer more flexibility, a modified sample clean-up and fractionation procedure is proposed for the use of the existing automated system DexTech. With the DexTech system it was possible to use different column settings to determinate dioxins/PCB and PBBE in different food and feedstuffs.

Methods and Materials

Samples:

In order to evaluate the suitability of the method for real sample analysis, a comparison between our well established manual clean-up procedure and two different column setting (Florisil or alumina column) automated system was made with several different food and feed matrices.

Reagents:

Native and ^{13}C-labelled PCDD/F and PCB, native PCDD/F, dioxin-like PCB (DL-PCB), indicator PCB (NDL-PCB) and PBBE standards were purchased from Promochem, Germany

Solvents used were of quality grade “Nanograde” and purchased from Promochem, Germany

Apparatus:

GC-HRMS: Agilent HP 6890/Micromass AutoSpec Ultima HRMS

GC-MS/MS: Agilent 7890 GC/Agilent 7000 Triple Quadrupole GC/MS/MS

DexTech system from LCTech Germany with two differnet column setting:

Setting 1:Sulphuric acid column, Florisil column und active carbon column

Setting 2: Sulphuric acid column, alumina column und active carbon column

Extraction procedures:

Different amounts of food or feeding stuff are mixed with sodium sulfate, placed into a glass fiber cartridge and extracted. The extraction takes place in a Soxhlet extractor with toluene/acetone 70/30 for 4-5 hours. Feeding stuffs are fortified with internal standards prior to the extraction. For food, the internal standards are added to the extracted fat.

Automatic clean-up with DexTech system from LCTech Germany

The extracted samples are resolved in 10 ml n-hexane and loaded directly into the sample loop of the system. For the setting 1 with Florisil column an additional adding of 2 ml toluene is necessary. In the other case with the setting 2 the absence of toluene is important not to destroy the retention of the alumina column. So the toluene form the extraction procedure must be evaporated very carefully. If the extracted material contains no fat it is advisable to add a keeper like nonane. The ready-to-use LCTech columns (acid silica, Florisil or alumina column and activated carbon column) are unpacked and placed into the column holder. The system starts with a conditioning step of the columns, injects the samples automatically and collects the fractions per sample. With the Florisil setting it is possible to separate the dioxins and the PCB and to collect three different fractions (PCDD/F; non-ortho PCB and the mono-

and di-ortho PCB including BDE). With the alumina setting the PCDD/F and the non-ortho PCB were collected together. At booth settings dichloromethane/n-hexane 1:1 is used to eluate the PCDD/F/PCB from the second columns (Florisil or alumina) and transfer them to the carbon column. This provided a better clean-up of the PCDD/F and non-ortho PCB fraction than to use only
n-hexane for the purification. The automated separation processes are shown in figure 1 and 2.

GC/MS Analysis:
a) GC-HRMS: Agilent 6890 GC/Micromass Autospec Ultima HRMS
PCB: Injector: 275°C, 1 µl splitless; Column: DB-5MS (J&W) 30 m, 0.25 #m film thickness, 0.25 mm ID; Temperature program: 80°C (3 min) - 175°C (30°C/min) - 270°C (3°C/min)
PCDD/F: Injector: 280°C, 1 µl splitless; Column: DB-5Dioxin (J&W) 60 m, 0.15 #m film thickness, 0.25 mm ID; Temperature program: 75°C (3 min) - 195°C (15°C/min) - 270°C (3°C/min)
BDE: Tri –Hepta: Injector: 275°C, 1 µl splitless; column: Agilent DB-5MS (J&W) 30 m, 0.10 #m film thickness, 0.25 mm ID; Temperature program: 100°C (3 min) - 330°C (10°C/min) - 330°C (4 min)
Deca: Injector: 275°C, 1 µl splitless ; column: Agilent DB-5MS (J&W) 15 m, 0.10 #m film thickness, 0.25 mm ID; Temperature program: 80°C (1,5 min) - 320°C (20°C/min) - 320°C (4 min)
Carrier gas: helium, pressure: 2 bar; MS-Resolution: 10000
b) GC-MS/MS: Agilent 7890 GC/ Agilent 7000 Triple Quadrupole MS
PCB: Injector: Multimode 100 °C, 2 µl splitless, up to 300 °C; Column: Agilent ZORBAX HT-8 Column 50 m × 0.22 mm, 0.25 µm film thickness, temperature program: Mono-ortho PCB: 80 °C (3.0 min hold), 20 °C/min to 160 °C, (0 min), 4 °C/min to 300 °C (8 min), (Total run time = 50.0 minutes); Non-ortho PCB: 120 °C (2.0 min hold), 40 °C/min to 160 °C (0 min), 7 °C/min to 300 °C (10 min), (Total run time = 33.0 minutes); MS transfer line temperature 280 °C
PCDD/F: Injector: Multimode 100 °C 2 µl splitless, up to 300 °C; Columns: Column (1) 2.0 m x 0.25 mm uncoated siltek deactivated fused silica, Column (2) Agilent J&W DB-5MS UI 60 m × 0.25 mm, film thickness 0.25 µm
Back flush time 15.0 minutes after injection; Oven program 130 °C (2.0 min hold), 10 °C/min to 200 °C, (16 min), 5 °C/min to 235 °C (7 min), 5 °C/min to 350 °C; MS transfer line temperature 300 °C
MS Condition: Electron energy –70 eV (PCDD/F) or –78 eV PCB; MS1 resolution: unit; MS 2 resolution: wide; Collision cell gas flows nitrogen at 1.5 mL/min, helium at 2.25 mL/min, MS temperatures ion source 280 °C, quadrupoles 150 °C, MRM settings were published previously. [1],[2]

Results and discussion
Routine chemicals and procedures for PCDD/F/PCB and PBBE analysis have been described elsewhere. [1],[2],[3]
With the DEXTech™- system including a Florisil column we are working since 2012 and have achieved good results over a long period. The system is used in our institute in the routine and we have participated in many proficiency tests and have passed all of them up to now. Nevertheless, in some cases the first fraction of the Florisil system required an improved purification. Therefore, an alumina column was tested with the flexible DEXTech™- system as known from a previous publication [4]. Also the possibility to use only one carbon column was tested. The new carbon column is also applicable to the Florisil system. Using the smart sulfuric acid both systems consume column only around 150 ml of solvent (table 1 and 2). A point that was repeatedly discussed during development is the replacement of dichloromethane (DCM). This is certainly possible, but the results show that flushing the carbon column only with n-hexane caused in some samples (e.g. compound feed) problems with the cleanness of the PCDD/F and non-ortho PCB extracts.

Using the large multilayer sulfuric acid column, including silver nitrate, the time and solvent consumption of the first n-hexane step duplicate. With this column it is possible to destroy up to 5 g fat (depending on the sample even more). With the Smart column (multilayer sulfuric acid column) samples with 1-2 g fat can be processed. Which columns can be used with different samples are a matter of experience and usually depends not only on the fat content of the sample. In figure 1 and 2 you can see the different column arrangements and the corresponding flow scheme; table 1 and 2 show the solvent consumption of the fractionation without conditioning. The conditioning is optional.

The manual clean-up running in our laboratory for decades provides good recoveries for the compounds. The comparison between DexTech setting 1 and 2 showed that the recoveries for all analyzed samples

were below 120 % and for nearly all samples over 60 % (table 3). In some cases, the recoveries were below 60 %, which is due to matrix interferences and can also be seen with the manual sample preparation. The shown recoveries are not only based on the sample clean up. They also include the sample extraction and the measurement. In comparison to the manual sample preparation, it can be said that the recoveries and the results are very robust with both automated DEXTech sample clean up settings.
The first setting with the Florisil column has the advantage, that the dioxins and PCBs are completely separated. In the second setting with the alumina column the dioxins cannot be separated from the non-ortho PCB and thus must be measured together. The non-ortho PCB elute earlier or with the TeCDD/F and also have concentrations that are higher two orders of magnitude than the PCDD/F. The benefit of the alumina column is to provide a cleaner fraction 1, which contains the mono-/di-ortho-PCB and BDE. It is important for the alumina system to rinse it with sufficient n-hexane. The dioxins and PCBs are indeed eluted after 70 ml or in 10 min from the Smart column. However, the alumina column should be flushed 5 times of the column volume with n-hexane again, otherwise interfering substances end up in the other fractions. The alumina and Florisil column are eluted via backflush with n-hexane/DCM to the carbon column. For this only very little solvent is needed (3 times of the column volume). The substances PCDD/F and non-ortho PCBs are retained on the carbon column. So there is a secondclean-up. In this way, very clean fractions for dioxins and non-ortho PCBs are obtained.

Conclusion:
The two column settings for the DEXTech system show a good performance for the clean-up of different feed and food samples for the analysis of PCDD/F, PCB and BDE. The development of new automatic clean-up systems with lower solvent consumption and a faster throughput will continue. The realization of a robust system that is able to process any sample and not only clear and pre-purified extracts, as well as the possibility of using different purification and separation principles in one system, are advantageous.

References:
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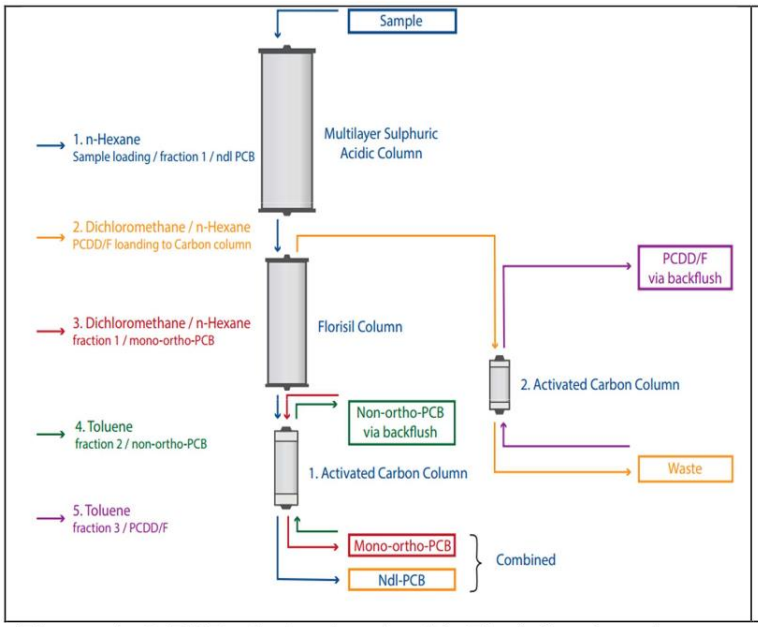

Figure 1: DEXTech (setting 1 with Florisil column)

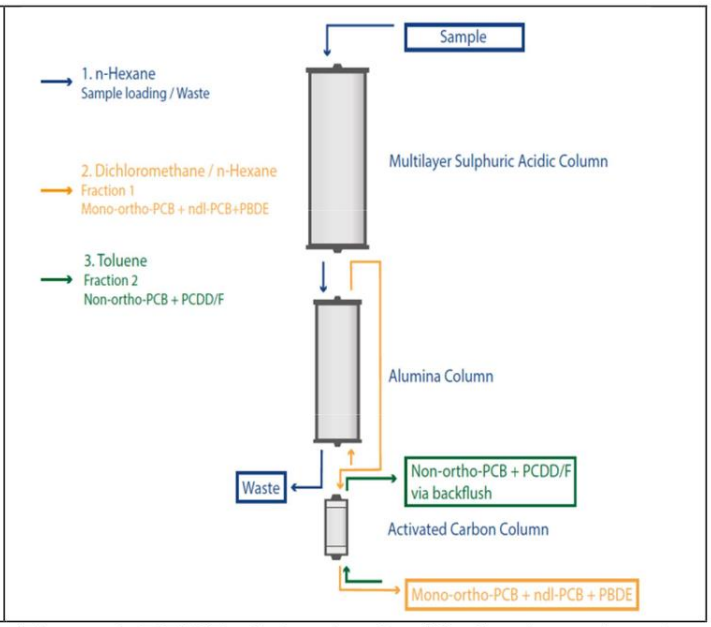

Figure 2: DEXTech (setting 2 with alumina column)

			minute	*flow ml*
fractionation				
S1	n-hexane	forward fraction 1	2	7
S1/S2/S3	n-hexane	forward fraction 1	1	7
S1/S2/S3	n-hexane	fraction 1	10	7
S2/S4	DCM/n-hexane	forward fraction 4	6	3
S3	DCM/n-hexane	fraction 2	5	3
S3	toluene	fraction 3	8	1
S4	toluene	fraction 4	10	2

solvent	**volumn**
n-hexane	91
DCM/n-hexane	33
toluene	28
sum	152
Time: 42 min	

Table 1: Time and Solvent consumption of the DEXTech (Setting 1 with Florisil as a second column)

			minute	*flow ml*
fractionation				
S1	n-hexane	forward fraction 1	2	7
S1/S2	n-hexane	forward fraction 1	14	7
S2/S3	DCM/n-hexane	fraction 1	8	3
S3	toluene	fraction 2	10	1

solvent	**volumn**
n-hexane	112
DCM/n-hexane	24
toluene	10
sum	146
Time: 34 min	

Table 2: Time and solvent consumption of the DEXTech (Setting 2 with alumina as a second column)

	Setting 1		Setting 2			Setting 1		Setting 2	
	recovery [%]	SD [%]	recovery [%]	SD [%]		recovery [%]	SD [%]	recovery [%]	SD [%]
2,3,7,8-TCDF	85	10	84	7	PCB-#28	88	28	83	12
1,2,3,7,8-PeCDF	89	10	86	10	PCB-#52	90	25	80	11
2,3,4,7,8-PeCDF	82	9	90	8	PCB-#101	95	29	82	13
1,2,3,4,7,8-HxCDF	86	20	88	10	PCB-#123	82	18	81	12
1,2,3,6,7,8-HxCDF	84	15	85	10	PCB-#118	78	19	79	13
2,3,4,6,7,8-HxCDF	76	15	84	10	PCB-#114	89	12	86	12
1,2,3,7,8,9-HxCDF	79	25	72	9	PCB-#105	71	30	84	13
1,2,3,4,6,7,8-HpCDF	84	8	77	11	PCB-#153	98	5	81	13
1,2,3,4,7,8,9-HpCDF	85	14	73	9	PCB-#138	92	11	84	12
1,2,3,4,6,7,8,9-OCDF	76	14	67	11	PCB-#167	81	19	80	13
2,3,7,8-TCDD	81	6	81	7	PCB-#156	78	24	83	13
1,2,3,7,8-PeCDD	89	16	85	9	PCB-#157	67	29	82	13
1,2,3,4,7,8-HxCDD	86	16	94	10	PCB-#180	99	16	83	12
1,2,3,6,7,8-HxCDD	89	13	84	11	PCB-#189	88	24	79	12
1,2,3,7,8,9-HxCDD	81	18	94	11					
1,2,3,4,6,7,8-HpCDD	92	16	78	11	BDE-#28	70	10	91	8
1,2,3,4,6,7,8,9-OCDD	78	17	74	13	BDE-#47	82	20	103	20
					BDE-#99	90	28	106	21
					BDE-#100	103	10	94	8
					BDE-#153	85	13	79	8
PCB-#077	83	7	91	19	BDE-#154	79	20	70	19
PCB-#081	83	7	91	19	BDE-#183	82	15	76	11
PCB-#126	88	5	103	15	BDE-#209	69	45	71	25
PCB-#169	78	8	108	14	PBB-#153	101	23	70	21

Table 3: Recoveries of the two DEXTech settings (Florisil (1) and alumina (2)) and their standard deviation (SD); Setting 1: N = 173; Setting 2 N = 24 (by setting two we worked with different active carbon concentration)