DETERMINATION OF PCDD/F AND PCB WITH A NEW AUTOMATED APPROACH FOR FAST SAMPLE PREPARATION AND MEASUREMENT WITH GC-HRMS AND GC-MS/MS

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Introduction

The major PCDD/F and PCB contamination cases which have occurred in the food and feed chain during the recent years illustrate the need for fast and high throughput methods to identify and confirm non-compliant samples and to trace back the contamination sources. For this purpose, a highly efficient clean-up procedure is required to purify raw extracts prior to the final analytical separation and quantification. This paper describes a new method which focusses on the automation of our well-proven sample clean-up procedure based on sulfuric acid coated silica gel, Forisil and carbon columns with the automated DECS System (LCTech). The fractionation process performed with the LCTech automated clean-up system enables a rapid and separate analysis of PCDD/F, non-ortho, and mono-ortho and di-ortho PCB. Gas chromatography with high resolution mass spectrometry (GC–HRMS) or gas chromatography with tandem mass spectrometry (GC-MS/MS) are used for the analytical determination of the three fractions. A comparison study was performed on quality-control samples and food samples of animal origin to evaluate the robustness of the new automated sample clean-up system compared to our standard method and also the quantification by GC-HRMS and GC-MS/MS. All results demonstrate the suitability of the automated LCTech sample preparation system and the GC-MS/MS system for a fast and reliable routine analysis of PCDD/F and PCB congeners in foodstuffs and feedstuffs that meet the requirements of European Union legislation.

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 the new automated system was made with several different food and feed matrices, including materials from the EURL proficiency test "Feed Fat 2013".

Reagents:

Native and ^{13}C-labelled PCDD/F and PCB, native PCDD/F, dioxin-like PCB (DL-PCB) and indicator PCB (NDL-PCB) 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

DECS system from LCtech Germany

Extraction procedures:

Different amounts of food or feeding stuff are mixed with sodium sulfate, placed into a glass fiber cartridge and extracted. Feeding stuff are fortified with internal standards prior to extraction. For food, the internal standards are added to the extracted fat. The extraction takes place in a Soxhlet extractor with toluene/acetone 70/30 for 16 hours overnight.

Manuel clean-up on a sulfuric acid column

After evaporation of the solvents, the extracts are cleaned up in a chromatography column filled with silica gel coated with sulfuric acid (44 %). PCDD/F and PCB are eluted with n-hexane.

Clean up with Florisil:

After evaporation of the solvents, the extracts 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 sulfate on top. The first eluate with n-hexane contains inter alia PCBs (F I). The PCDD/F elution is performed with toluene (F II).

Clean up of the n-hexane fraction (F I) with active carbon for DL-PCB and NDL-PCB:

Celite 545 and Norit (10:1) are mixed thoroughly and 0.1 g of this mixture is filled into a chromatography column. The column is washed with 50 ml toluene and 50 ml dichloromethane/cyclohexane (1/1). The n-hexane extract from the Florisil clean-up (F I) is dissolved in 0.5 ml n-hexane and applied onto the column. The first eluate of 50 ml dichloromethane/cyclohexane (1/1) contains the mono-ortho PCB and NDL-PCB. After concentration to 3 ml and addition of a syringe spike (^{13}C-1.2.3.4-TCDD) and 10 µl dodecane, the extract is carefully evaporated to dryness under a gentle stream of nitrogen and finally reconstituted with 15 µl toluene. The reconstituted solution is used for GC-HRMS and GC-MS/MS analysis of mono-ortho and NDL-PCB. The non-ortho PCB elution is performed with toluene. After addition of a syringe spike, the extract is evaporated under a gentle stream of nitrogen, reconstituted with 40 µl toluene and analysed with GC-HRMS and GC-MS/MS.

Clean-up of the toluene fraction (F II) with active carbon for PCDD/F:

Carbopack C (18 %) and Celite 545 (82 %) are mixed thoroughly and 0.25 g of the mixture are filled into a chromatography column. The column is 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 from the Florisil clean-up (F 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/F elution is performed with toluene. After addition of a syringe spike, the extract is carefully evaporated under a gentle stream of nitrogen, reconstituted with 12 µl toluene and transferred into an auto sampler vial for GC-HRMS and GC-MS/MS analysis.

Automatic clean-up with a DECS system from LCTech Germany

The extracted samples are resolved in 10 ml n-hexane and loaded directly into the sample loop of the system. The ready-to-use LCTech columns (acid silica, Florisil and two activated carbon columns) are unpacked and placed into the column holder. The system starts with a conditioning step of the columns, injects the samples automatically and collects three fractions per sample. The automated separation process follows the same principle as the manual clean-up. The whole sample clean-up takes 97 minutes. Half of the time is needed for conditioning the columns.

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 µm film thickness, 0.25 mm ID; Temperature programme: 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 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

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 programme: 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 programme 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

The aim was to develop an automatic clean-up system for PCDD/F and PCB analysis based on our manual procedure which has been used for many years in our institute. The manual sample preparation has well-proven in numerous proficiency tests and was applied in many food and feed crisis cases during the past 25 years. It is reproducible, fast for a manual preparation procedure and meets the requirements of European Union legislation. In co-operation with the company LCTech/Germany, it was possible to automate the well-proven method to generate the same valid and reproducible results in less time. The principle of the method is based on the clean-up of the acid stable PCDD/F and PCB on silica gel coated with sulfuric acid. A separation of the PCDD/F from PCB is subsequently performed on a Florisil column. For further purification, both eluats of the Florisil column are cleaned up on two different carbon columns which contain a different active carbon. The PCB fraction can be split into a group of non-ortho PCB, and a fraction containing the mono- and di-ortho PCB. This is important because the non-ortho PCB fraction includes PCB 126 and 169 which were assigned the highest toxicity factors of the PCB (WHO 2005). If PCB 126 is measured along with the other PCB, it may cause interferences, which can lead to a substantial overestimation of the PCB 126 concentration depending on the separation column. It is therefore essential to separate the non-ortho PCB from the other PCB. If one wants to avoid a third sample run, the non-ortho PCBs can be measured together with the PCDD/F. The PCDD/F fraction also needs to be cleaned up on a carbon column to separate matrix substances which may potentially interfere especially with the tetra-, penta- or hexa-CDD/F traces in MS analysis.

The advantage of the automated clean-up procedure is that the columns are easy to handle and can be bought filled and ready-to-use. The whole process of column conditioning and clean-up of the sample extracts is done automatically in 97 minutes. Through the automatic sample clean-up in one step one saves time and reduces potential losses of PCDD/F and PCB congeners during solvent evaporation after each manual column clean up step.

Next to the analysis of different feed and food samples we tested the performance of the system by analysing the proficiency test (PT) material “Feed Fat 2013” from the EURL for Dioxin and PCB, Freiburg. The recoveries for each congener determined in the PT material with the automated sample preparation system are shown in Table 1. In all experiments, the percentages of the PCDD/F and PCB recoveries are in good agreement with the legislation requirements and range between the requested limits of 60 % to 120 %.

	recovery mean (%)	standard diviation (%)		recovery mean (%)	standard diviation (%)
2,3,7,8-TeCDD	82	10	PCB 77	82	9
1,2,3,7,8-PeCDD	91	10	PCB 81	82	10
1,2,3,4,7,8-HxCDD	94	18	PCB 126	92	13
1,2,3,6,7,8-HxCDD	93	14	PCB 169	90	8
1,2,3,7,8,9-HxCDD	96	16	PCB 105	80	10
1,2,3,4,6,7,8-HpCDD	91	18	PCB 114	98	15
OCDD	88	14	PCB 118	85	17
2,3,7,8-TeCDF	73	11	PCB 123	91	14
1,2,3,7,8-PeCDF	86	13	PCB 156	88	17
2,3,4,7,8-PeCDF	82	12	PCB 157	79	17
1,2,3,4,7,8-HxCDF	95	15	PCB 167	93	18
1,2,3,6,7,8-HxCDF	96	19	PCB 189	87	19
2,3,4,6,7,8-HxCDF	83	13	PCB 28	99	13
1,2,3,7,8,9-HxCDF	84	17	PCB 52	98	14
1,2,3,4,6,7,8-HpCDF	90	16	PCB 101	90	14
1,2,3,4,7,8,9-HpCDF	84	14	PCB 138	94	14
OCDF	84	14	PCB 153	103	8
			PCB 180	88	19

Table 1: recovery and standard deviation for each congener in the proficiency test material; n=9, sample intake from 1 to 5 g

Table 2 shows the assigned values (Huber robust mean) and the robust standard deviations reported by the organizer of the EU proficiency test "Feed Fat 2013". These data are compared with the results of a 9-fold analysis of the sample. For this, the feed fat samples were analyzed with the automated sample clean-up method. A z-score was calculated for each of the nine samples ((result - assigned value) / standard deviation). As a result, all parameters are very close to the assigned values. The average of the calculated z-scores are in the range of -1 to +1. The sample weights of 1-5 g seem to have no impact on the quality of the results. The sulfuric acid column is designed for a capacity of at least 5 gram of fat. The results confirm that even with a sample weight of 5 g fat no negative effects on the recoveries or the calculated results are obtained.

	EURL assigned value (Huber robust mean) ng/kg produkt (12 % moisture content)	EURL robust standard diviatio; ng/kg produkt (12 % moisture content)	mean of calculated z-Score of each analysed sample (n=9)		EURL assigned value (Huber robust mean) ng/kg produkt (12 % moisture content)	EURL robust standard diviatio; ng/kg produkt (12 % moisture content)	mean of calculated z-Score of each analysed sample (n=9)
2,3,7,8-TeCDD				PCB 77	85,2	8,34	-1,26
1,2,3,7,8-PeCDD				PCB 81	2,63	0,47	0,97
1,2,3,4,7,8-HxCDD				PCB 126	18	2,1	-0,07
1,2,3,6,7,8-HxCDD				PCB 169			
1,2,3,7,8,9-HxCDD				PCB 105	546	53,4	-0,81
1,2,3,4,6,7,8-HpCDD	0,255	0,0434	0,72	PCB 114	30,1	6,09	-1,38
OCDD Octachlordibenzodioxin	2,42	0,318	0,48	PCB 118	990	96,8	0,13
2,3,7,8-TeCDF	0,306	0,0381	-0,21	PCB 123	22,1	3,68	-1,14
1,2,3,7,8-PeCDF	0,132	0,0284	0,04	PCB 156	136	11,9	-0,07
2,3,4,7,8-PeCDF	0,139	0,032	0,98	PCB 157	34,5	5,15	0,19
1,2,3,4,7,8-HxCDF	0,142	0,0253	1,70	PCB 167	53,3	7,02	0,04
1,2,3,6,7,8-HxCDF	0,0869	0,0254	-0,23	PCB 189	7,01		
2,3,4,6,7,8-HxCDF				WHO-PCB-TEQ (WHO-TEF 2005) upper bound	1,89	0,213	-0,09
1,2,3,7,8,9-HxCDF				PCB 28			
1,2,3,4,6,7,8-HpCDF	0,197	0,0385	0,37	PCB 52	0,251	0,0348	1,07
1,2,3,4,7,8,9-HpCDF				PCB 101	0,629	0,0778	-0,02
OCDF Octachlordibenzofuran				PCB 138	0,966	0,146	-0,98
WHO-PCDD/F-TEQ (WHO-TEF 2005) upper bound	0,206	0,0444	0,95	PCB 153	0,749	0,0855	-0,68
WHO-PCDD/F-PCB-TEQ (WHO-TEF 2005) upper bound	2,17	0,254	-0,24	PCB 180	0,245	0,0361	0,13
				Sum ndl PCB	3,16	0,46	-0,88

Table 2: EURL Proficiency test in Feed Fat 2013: comparison of the reported assigned value by the EURL for Dioxin and PCB and the mean
of the calculated z-sores ((result – assigned value)/standard deviation) of each analyzed sample (n=9); sample intake from 1 to 5 g

The extracts from the manual and automatic clean up were both measured with GC-HRMS and with a GC-MS/MS system. The results (not shown) are comparable with the results we published previously[3] and they are in the range of ±20 %.

Conclusion

A comparison of analytical results obtained for the PT material "Feed Fat 2013" by GC-HRMS and GC-MS/MS with manual or automatic sample preparation demonstrates the suitability of the DECS LCTech system and the Agilent 7000 Triple Quadrupole GC-MS system for a fast and reliable routine analysis of PCDD/F and PCB

congeners in foodstuffs and animal feed at the level of interest that meet the requirements of European Union legislation.

References

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