GC-MS/MS DETERMINATION OF PCDD/FS AND PCBS IN FEED AND FOOD - COMPARISON WITH GC-HRMS

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Introduction

Methods of sampling and analysis for the official control of levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) for feedingstuff and food are defined in. Commission Regulation (EC) No 152/2009 and Commission Regulation (EC) No 1883/2006 respectively. For the analysis of PCDD/Fs and dioxin-like PCBs (DL-PCBs) in food and feed screening and confirmatory methods can be applied[1,2]. Screening methods can comprise e.g. GC-MS, GC-MS/MS methods or bioanalytical screening methods, confirmatory methods are defined as GC-HRMS methods.

The objective of this evaluation is to check the ability of GC-MS/MS systems for analyzing PCDD/Fs and DL-PCBs in feed and food at the level of interest. Important criteria for application of a GC/MS method for screening and possibly also for confirmation are:

- availability of applicable detection methods,
- criteria for identification and
- definition of working range and limit of quantification (LOQ)[5,6].

Materials and Methods

Extraction and Clean-up

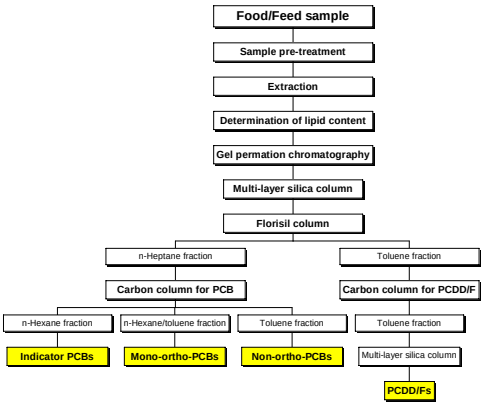

Figure 1: Extraction and clean-up for determination of PCDD/Fs and PCBs in food and feed samples

The extraction and clean-up process for food and feed samples was performed according figure 1 for GC-MS/MS and GC-HRMS. The identical extracts were measured on both instruments.

For food samples with legal limits on fat basis, the application of a maximum of 3 g of fat for clean-up is the limiting factor for achieving low limits of quantification in this method.

GC-MS measurement

The GC-MS/MS measurements were performed using a TSQ Quantum XLS Ultra Triple-Quadrupole GC-MS/MS provided by Thermo Scientific, Austin, USA.

The following MS/MS settings were applied:

Source temperature	250 °C
Ionization	EI
Electron energy	40 eV
Emission current	50 µA
Q2 Gas pressure (Argon)	1.5 mTorr
Collision Energy	22 V
Q1 peak width	0.7 amu
Q3 peak width	0.7 amu

Precursor ion	M+
Product ion	M-COCl+
2 precusor ions, each with 1 product ion	

The Q2 gas pressure and collision energy were optimized for PCDD/F measurement.

PTV and GC settings were identical for GC-MS/MS and GC-HRMS. For GC-MS/MS only the final oven temperature had to be adjusted to 285 °C (GC-HRMS: 340 °C):

PTV injection (PCDD/Fs)	
Injected volume	5 µl (toluene)
Injection speed	5µl/s
Liner	Open silcosteel liner
Injection temperature	100 °C
Transfer temperature	340 °C

GC programme (PCDD/Fs)	
GC column	DB-5MS (60m, 0.25 µm, 0.25 mm)
Initial temperature	120 °C
Rate 1	17°C/min to 250 °C
Rate 2	2.5°C/min to 285 °C
Final temperature	285 °C for 13 min

The results of the GC-MS/MS measurements were compared with routine GC-HRMS measurements using DFS High Resolution MS (Thermo Scientific, Bremen, Germany).

Calibration

For calibration of the GC-MS/MS and GC-HRMS system the following calibration solutions (table 1) were applied. For checking of the performance of the GC-MS/MS system in the low concentration range additionally 1:2 and 1:5 dilutions of the lowest calibration point were measured. The calculated WHO-PCDD/F-TEQ for these calibration solutions and dilutions, based on the analysis of 3 g of fat and an injection of 5 µl out of 20 µl final volume, ranged between 0.12 and 24 pg/g fat.

Table 1: Concentrations of individual congeners in calibration solutions for GC-MS/MS and GC-HRMS

Conc. in pg/µl	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5
2,3,7,8-TCDD	0.0125	0.025	0.05	0.2	0.5
1,2,3,7,8-PeCDD	0.025	0.05	0.1	0.4	1
1,2,3,4,7,8-HxCDD	0.025	0.05	0.1	0.4	1
1,2,3,6,7,8-HxCDD	0.0625	0.125	0.25	1	2.5
1,2,3,7,8,9-HxCDD	0.025	0.05	0.1	0.4	1
1,2,3,4,6,7,8-HpCDD	0.125	0.25	0.5	2	5
OCDD	0.25	0.5	1	4	10
2,3,7,8-TCDF	0.0125	0.025	0.05	0.2	0.5
1,2,3,7,8-PeCDF	0.0125	0.025	0.05	0.2	0.5
2,3,4,7,8-PeCDF	0.0625	0.125	0.25	1	2.5
1,2,3,4,7,8-HxCDF	0.025	0.05	0.1	0.4	1
1,2,3,6,7,8-HxCDF	0.025	0.05	0.1	0.4	1
1,2,3,7,8,9-HxCDF	0.0125	0.025	0.05	0.2	0.5
2,3,4,6,7,8-HxCDF	0.0125	0.025	0.05	0.2	0.5
1,2,3,4,6,7,8-HpCDF	0.025	0.05	0.1	0.4	1
1,2,3,4,7,8,9-HpCDF	0.0125	0.025	0.05	0.2	0.5
OCDF	0.05	0.1	0.2	0.8	2

Results and Discussion

Ion abundance ratio

One important criteria for the identification of the PCDD/F congeners is the ion abundance ratio between the 2 monitored product ions, resulting from 2 different precursor ions. This ratio depends on the ion abundance ratio of the selected precursor ions and the probability of the loss of $CO^{35}Cl$ or $CO^{37}Cl$ for formation of each product ion. The measured ion abundance ratios in calibration matched the calculated theoretical values within the QC limits of ± 15 %.

Calculation of the limit of quantification

Due to the very low noise level in the GC-MS/MS system, the calculation of an LOQ from the signal-to-noise ratio was not possible. Therefore, the LOQ was calculated from the lowest concentration with acceptable signal-

to-noise-ratio, ion abundance ratio (within ± 15 % of theoretical value) and deviation of the relative response factor from the mean value (CV ≤ 20%, maximum deviation of individual congeners of mean value ≤ 40 %). From the measurement of 10 GC-MS/MS sequences the following range of LOQs was calculated for the individual congeners:

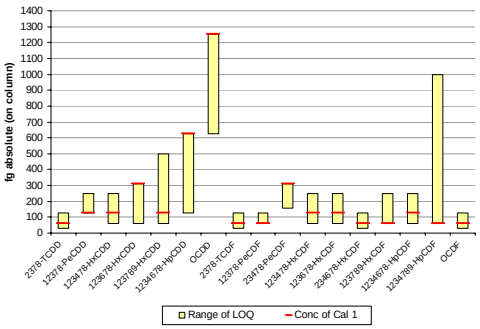

Figure 2: Range of limit of quantification calculated from lowest calibrated level

Comparison with GC-HRMS results and spiked concentration

For comparison of the results of GC-MS/MS and GC-HRMS measurements, the deviation of the results of the GC-MS/MS measurements from GC-HRMS were calculated. For different food samples and human milk, covering a concentration range between 0.1 and 10 pg WHO-PCDD/F-TEQ/g fat, deviations of the GC-MS/MS results lay below 20 % in most cases. In the concentration range below 0.5 pg/g fat considerably higher deviations could be observed due to the higher limits of quantification (LOQ) calculated from the respective calibration of the GC-MS/MS systems. In some cases higher deviations of the GC-MS/MS results of GC-HRMS could also be observed in the range of low maximum levels defined for food of animal origin on fat basis (e.g. for pork). The differences between upper and lower bound calculation of the TEQ were mostly below 20 %, with higher deviations for the low concentration range and some samples in the range of 2 pg/g fat due to higher LOQs for these samples (figure 3).

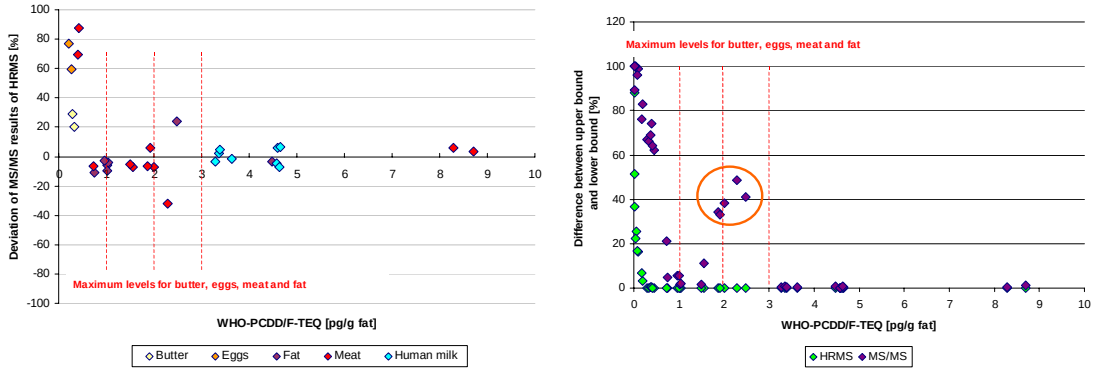

Figure 3: Deviations of GC-MS/MS results of GC-HRMS (%) and differences between upper and lower bound WHO-PCDD/F-TEQ calculation (%)

Comparable results could be found for the comparison of the GC-MS/MS results with spiked concentration covering a concentration range up to 5 pg WHO-PCDD/F-TEQ/g fat.

For fish and feed with legal limits defined on fresh weight and product (12% moisture content) respectively, acceptable results we observed also in the concentration range considerably below legal limits due to the higher sample amount applicable for extraction and clean-up.

Modified clean-up

A modified clean-up with a reduced number of clean-up steps, skipping the carbon column for further cleaning of the PCDD/F fraction, was applied in order to make a first test of the robustness of the GC-MS/MS systems. The comparison between the normal and the reduced clean-up showed no significant differences between chromatograms and measured concentrations. Only some additional non interfering peaks occurred, but a increase of the noise was not observed. In the course of these tests, the influence of the adjustment of the Q1 and Q3 peak width on the results was checked. An increase of the peak width of Q1 from 0.4 amu to 2.0 amu resulted in higher peak intensity of the analytes of interest but also in an increasing noise level, especially for PCDFs. At a peak width of 2.0 amu at Q3 interfering traces of ^{13}C-labeled PCDFs were visible in the corresponding PCDD mass traces.

Conclusions

The GC-MS/MS system is in principle applicable for the PCDD/F analysis in food and feed samples. A good correlation between the results of GC-MS/MS and GC-HRMS could be observed for the concentration range above 1 pg WHO-PCDD/F-TEQ/g fat for food and human milk samples in most cases. Higher deviations of HRMS results occurred in the range below 1 pg WHO-PCDD/F-TEQ/g fat depending on limit of quantification and amount of fat applied for clean-up. For fish and feed samples acceptable deviations were also observed in the concentration range considerably below the established legal limits.

A calculation of limit of quantification (LOQ) from the signal-to-noise ratio, as defined in EU regulations and performed for the GC-HRMS, was not possible due to the low noise levels. Therefore the LOQ was calculated from the lowest calibrated level.

Further evaluation of the performance of the GC-MS/MS system will focus on the analysis of dioxin-like PCBs.

Acknowledgements

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References

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