Comparative study on the analysis of PCDD and PCDF in food and animal feed using GC-MSMS and GC-HRMS

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1. Introduction

Contaminations of food and feed with persistent organic pollutants (POP) are determined routinely by various analytical technologies. Dioxins and dioxin like substances belong to this category. They are regarded to have high degree of toxicity to humans. The majority of dioxin contamination of humans is done via the food chain. The current methods to determine the amount of dioxins and dioxin like substances is described in European legislations [1]. In the past mainly gas chromatography coupled to high resolution mass spectrometry with isotopic dilution has been used as analytical method for analyzing and quantifying dioxins. Since June 2014 the EU regulation also allows gas chromatography coupled to tandem quadrupole mass spectrometry (GCMSMS) as a confirmatory method [2]. Dioxins as referred to in this regulation cover a group of 75 polychlorinated dibenzo-para-dioxin (PCDD) congeners and 135 polychlorinated dibenzofuran (PCDF) congeners, of which 17 are of toxicological concern. There have been several publications where the suitability of GC-MS [3] or GC-MSMS [4] has been tested in the past. Based on those data the new EU regulation included GC-MSMS as an alternative for quantitative confirmation of dioxins and PCBs. In this application more than 50 samples of different matrices were split and analysed by the Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer and the Waters Autospec GC-HRMS*.

2. Experimental

Calibration standards of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans with appropriate ^{13}C isotope labelled internal standards were supplied by Greyhound chromatography (Wellington). ^{13}C labelled internal standards were spiked before sample preparation and used for quantification. Additionally, ^{13}C labelled recovery standards were added before instrumental analysis. Analysis was performed on GC-HRMS and GC-MSMS. Analytical conditions for GC-MSMS are summarized in Tab.1.

Table 1: Analytical Conditions

GC	
Instrument:	GCMS-TQ8040 (Shimadzu, Japan)
Injector:	SPL-2010 Plus
Injection Temperature:	280 °C
Split:	Splitless Injection (1min)
Injection Volume:	2 µL
Linear Velocity:	34.7 cm/sec
Column:	5 MS 60 m, 0.25 mm, 0.1 µm
GC Oven:	130 °C, 1min, 20 °C/min to 190 °C, 8 min, 2 °C/min to 220 °C, 3 min, 6 °C to 244 °C, 15 °C to 320 °C, 4 min
MS	
Transfer Line:	280 °C
Ion Source:	230 °C
Emission Current:	150 µA
Ionization Mode:	EI, 70 eV
Acquisition Mode:	MRM
CID Gas:	Argon (200 kPa)

3. Results

Fig.1 and Tab.2 summarize the results of the instrument calibration. Fig. 1 shows peak profiles at the lowest standard concentration and the corresponding calibration curve. The used calibration ranges for Tetra and Penta were between 0.1 pg/µl – 10 pg/µl, for Hexa and Hepta between 0.2 pg/µl – 20 pg/µl, and for OCDD and OCDF between 0.5 pg/µl – 50 pg/µl. Linearity of all calibration curves showed R^2-values higher than 0.999.

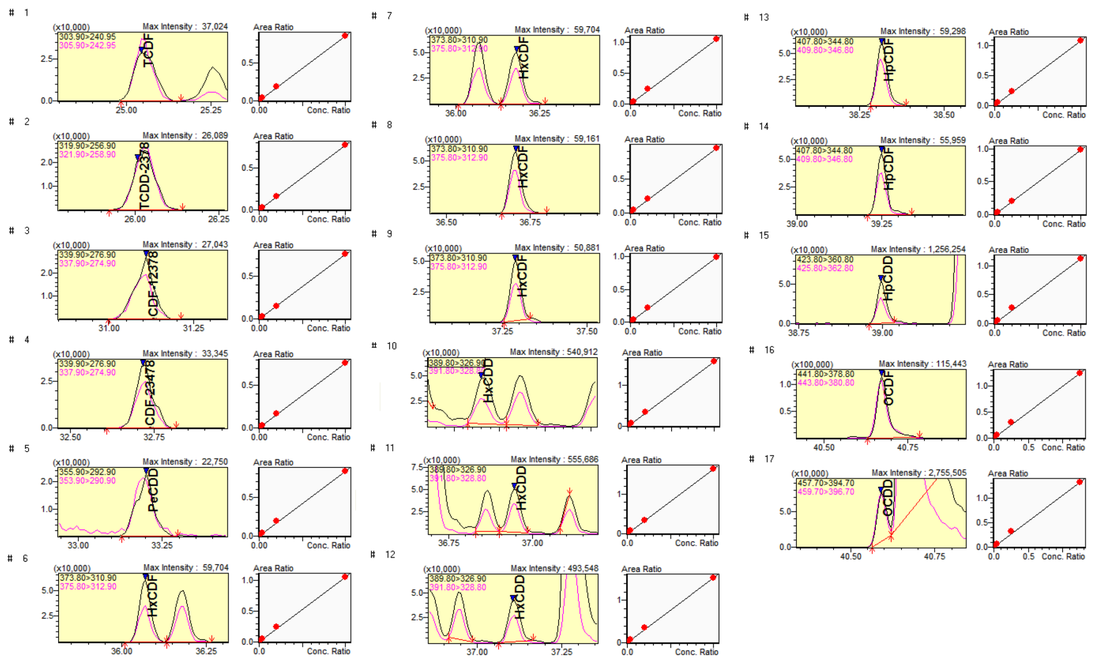

Fig.1: Peak Profiles at lowest standard concentration (Tetra/Penta: 0.1 pg/µl, Hexa/Hepta: 0.2 pg/µl, OCDD/OCDF: 0.5 pg/µl) and corresponding calibration curves (R^2 >0.999)

Eight replicates were done on the lowest standard. The RSD% was below 3%, which indicates in MDL of 16.78 fg with a confidence level of 99%. Tab.2 summarizes retention times, MRM transitions and collusion energies for the 17 target compounds.

Table 2: Retention Time, MRM transitions and collusion energies for 17 target congeners

	Ret.-Time	Quantifier	CE	Qualifier	CE
1. 2378-TCDF	25.04	303.9>240.9	33	305.9>242.9	33
2. 2378-TCDD	26.02	319.9>256.9	24	321.9>258.9	24
3. 12378-PeCDF	31.09	339.9>276.9	35	337.9>274.9	35
4. 23478-PeCDF	32.71	339.9>276.9	35	337.9>274.9	35
5. 12378-PeCDD	33.19	355.9>292.9	25	353.9>290.9	25
6. 123478-HxCDF	36.06	373.8>310.9	35	375.8>312.9	35
7. 123678-HxCDF	36.17	373.8>310.9	35	375.8>312.9	35
8. 234678-HxCDF	36.70	373.8>310.9	35	375.8>312.9	35
9. 123789-HxCDF	37.28	373.8>310.9	35	375.8>312.9	35
10. 123478-HxCDD	36.86	389.8>326.9	25	391.8>328.8	25
11. 123678-HxCDD	36.94	389.8>326.9	25	391.8>328.8	25
12. 123789-HxCDD	37.10	389.8>326.9	25	391.8>328.8	25
13. 1234678-HpCDF	38.31	407.8>344.8	36	409.8>346.8	36
14. 1234789-HpCDF	39.24	407.8>344.8	36	409.8>346.8	36
15. 1234678-HpCDD	38.99	423.8>360.8	25	425.8>362.8	25
16. OCDF	40.67	441.8>378.8	35	443.8>380.8	35
17. OCDD	40.59	457.7>394.7	26	459.7>396.7	26

Based on the calibration data the concentration for each congener is determined using ^{13}C-labelled internal standards. As each congener shows different toxicity, the WHO introduced in 2005 the toxic equivalent system (TEQ). This system assigns to each congener a specific toxic equivalent factor (TEF), which expresses the differences in toxicity. Multiplication of these factors with the individual concentration of each congener and final summation results in overall value, which can be seen as a measure of toxicity for the investigated sample. This sum parameter is called toxic equivalent (TEQ).

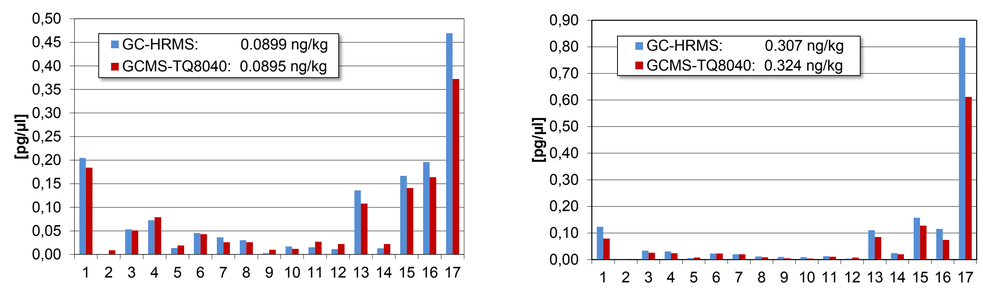

Fig.2: Comparison of concentrations (pg/µl) of individual PCDD and PCDF congeners determined for an animal feed (left) and fish sample (right). The x-axis numbers refer to the compounds listed in table 2.

In Fig. 2, a component based comparison for an animal feed and fish sample is shown. The TEQ values calculated from these samples were for the animal feed sample 0.0899 ng/kg (GC-HRMS) and 0.0895 ng/kg (GC-MSMS) and for the fish sample 0.307 ng/kg (GC-HRMS) and 0.324 ng/kg (GC-MSMS).

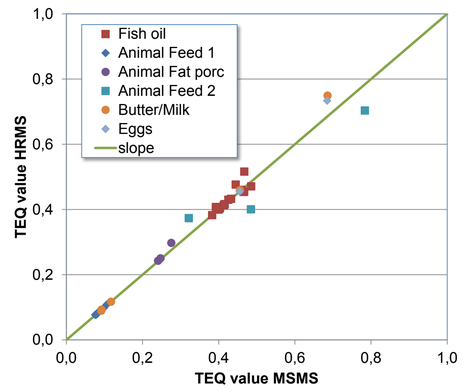

Fig.3: TEQ values (upper bound) in ng/kg calculated from GC-HRMS and GC-MSMS for various matrices

The described procedure was applied to more than 50 samples. In figure 3 TEQ values calculated from GC-HRMS and GC-MSMS data are plotted against each other for various matrices. In addition the ideal curve with slope 1 is shown as well.

4. Discussion

The TEQ values derived from the GC-MSMS methods shown above, indicate a very good correlation with the established HR-GCMS methods. For the matrix fish, the deviation is less than 10% at TEQ levels of about 0.45 ng/kg fat. Those values are below the regulatory levels which are 1.75 ng/kg (marine oil, fish oil). The highest TEQ value observed was about 10 ng/kg. The recovery of the compounds was calculated for every sample from the recovery internal standards and the results were between 60 and 100%.

5. Conclusion

The data shown in this application indicate that the GCMS-TQ8040 proves sufficient accuracy for quantitative confirmation of dioxins in food and feed samples. The maximum deviation of TEQ values calculated from GC-MSMS data compared to the one from GC-HRMS were below 10% for many matrices measured, even for low TEQ values below 0.5 ng/kg.

6. Literature

[1] Commission Regulation (EU) No 252/2012 & Commission Regulation (EC) No 278/2012
[2] Commission Regulation (EC) No 589/2014 & Commission Regulation (EC) No 709/2014
[3] M. Geissler, S. Schröder Lab&more 2/11, p20
[4] A. Kotz et al, Organohalogen Compounds Vol. 74, 156-159 (2012)

* Measurements were done at SGS Antwerp