

Application News

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GCMS

Quantitative Analysis of Dibenzo-p-dioxins (PCDD) and Polychlorinated-p-Dibenzofurans (PCDF) in Foodstuff and animal Feed using the GCMS-TQ8030 tandem mass spectrometer

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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. Dioxins are introduced via several ways into the food products. As an example eggs can be contaminated via the feed of hens. The current methods to determine the amount of dioxins and dioxin like substances is described in American and European legislations [1,2]. 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. 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. Polychlorinated biphenyls (PCBs) are a group of 209 different congeners which can be divided into two groups according to their toxicological properties: 12 congeners exhibit toxicological properties similar to dioxins and are therefore often termed dioxin-like PCBs (DL-PCBs). The other PCBs do not exhibit dioxin-like toxicity and have a different toxicological profile.

There have been several publications where the suitability of GCMS [3] or GCMSMS [4]

has been tested in the past. Based on those data the new EU regulation included GCMSMS 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-TQ8030 triple quadrupole mass spectrometer and the Waters Autospec GCHRMS.

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.

The schematic diagram of sample preparation is shown in figure 1.

For this application samples prepared and measured with HRMS were also analysed with the Shimadzu GCMS-TQ8030 tandem mass spectrometer1). The chromatographic column was a 5% phenyl with 60 m, 0.25 mm, 0.1 μm film MS column. Injection volume was 2 μl into the SPL-2010 in splitless mode. Each compound was measured with 2 MSMS transitions.

The mass spectrometer time settings are shown in table 1. Table 2 shows the target compounds and their internal standards which were measured together with one quantifier and one qualifier MSMS transition.

	start T	end T	Event Time (s)	MRM (Quant)	CE	MRM(Qual)	CE	
13C TCDF ISTD	20	28	MRM	0,01	315.95>251.95	33	317.95>253.95	33
TCDD-2378	20	28	MRM	0,3	319.90>256.90	24	321.90>258.90	24
13C TCDD-2378 ISTD	20	28	MRM	0,01	331.90>268.00	24	333.90>270.00	24
13C TCDD-1234 ISTD	20	28	MRM	0,01	331.90>268.00	24	333.90>270.00	24
TCDF	20	28	MRM	0,3	303.90>240.95	33	305.90>242.95	33
PeCDF-23478	28	35	MRM	0,3	339.90>276.90	35	337.90>274.90	35
13C PeCDF-23478 ISTD	28	35	MRM	0,01	351.90>287.90	35	349.90>285.90	35
PeCDD	28	35	MRM	0,3	355.90>292.90	25	353.90>290.90	25
13C PeCDD ISTD	28	35	MRM	0,01	365.90>301.90	25	367.90>303.90	25
HxCDF	35	38	MRM	0,2	373.80>310.90	35	375.80>312.90	35
13C HxCDF ISTD	35	38	MRM	0,01	385.80>321.90	35	387.80>323.90	35
HxCDD	35	38	MRM	0,2	389.80>326.90	25	391.80>328.80	25
13C HxCDD ISTD	35	38	MRM	0,01	399.90>335.90	25	401.80>337.90	25
HpCDD	38	40	MRM	0,2	423.80>360.80	25	425.80>362.80	25
13C HpCDD ISTD	38	40	MRM	0,01	435.80>371.80	25	437.80>373.80	25
HpCDF	38	40	MRM	0,2	407.80>344.80	36	409.80>346.80	36
13C HpCDF ISTD	38	40	MRM	0,01	419.80>355.90	36	421.80>357.90	36
OCDF	40	43	MRM	0,2	441.80>378.80	35	443.80>380.80	35
13C OCDF ISTD	40	43	MRM	0,01	453.80>389.80	35	455.80>391.80	35
OCDD	40	43	MRM	0,2	457.70>394.70	26	459.70>396.70	26
13C OCDD ISTD	40	43	MRM	0,01	469.80>405.80	26	471.80>407.80	26

Table 1: MRM table with measuring window (start T, end T), event time in seconds and transitions with collision energy CE. Total number of time segments is 5. The MS resolution was set to Q1: high/unit (depending on peak) and Q3: low

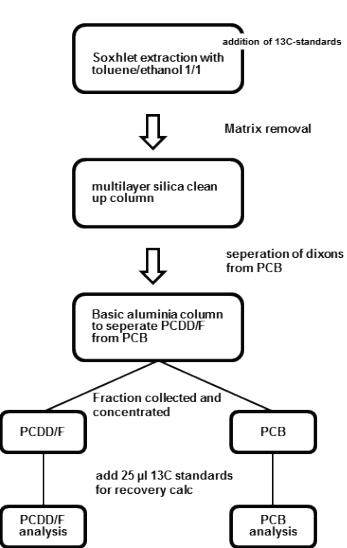


Figure 1: flow diagram of real world sample preparation

The other instrument parameters were: Splitless injection into a splitless liner (Shimadzu) at an injector temperature of 280 °C. GC oven temperature started at 130 °C for 1 min, 20 °C/min to 190 °C, 8 min, 2 °C/min to 220 °C, 3 min, 6 °C/min to 244 °C. The pneumatics were operated in the constant linear velocity mode at 34.7 cm/sec. Interface and ion source temperature were at 280 °C and 230 °C, respectively.

Results

In figure 2 the results recorded with a standard are shown. The target compounds are shown together with the calibration curves. The concentrations are: Tetra: 0.1 pg/µl, Penta, Hexa, Hepta: 0.2 pg/µl, Octa: 0.5 pg/µl.

The compounds with retention times and quantifier/qualifier transitions are shown in table 2.

The calibration ranges used were 0.1 pg/µl – 10 pg/µl for Tetra and Penta, 0.2 pg/µl – 20 pg/µl for Hexa and Hepta, 0.5 pg/µl – 50 pg/µl for OCDD and OCDF with $R^2 > 0.999$.

Eight replicates were done on the lowest standard. The RSD% was below 3%. The instrument detection limit is calculated from the following formula:

$$IDL = t_{\alpha n} n \cdot RSD_x (\text{amount standard}) / 100\%$$

$$t_{\alpha n} = 2.998 \text{ (student t table, } \alpha = 0.01 \text{ (99% confident level)}$$

The IDL calculated by that formula is 16.78 fg (Tetra, Penta).

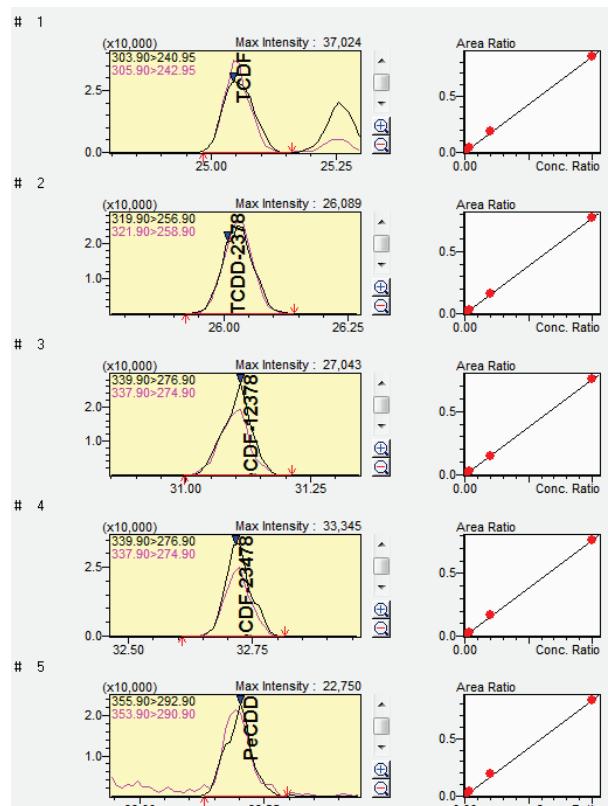
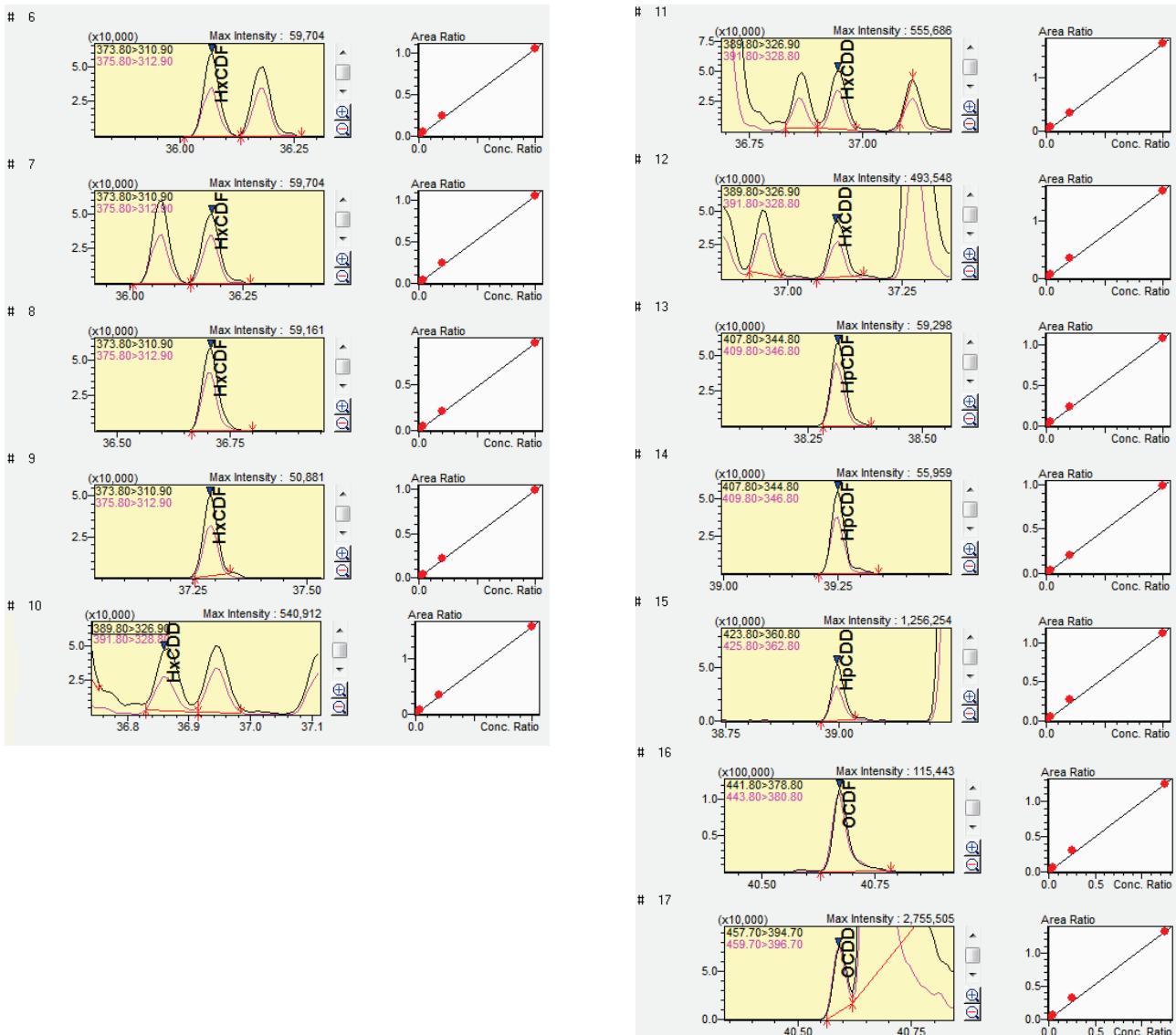


Figure 2: Native PCDD and PCDF congeners recorded with the lowest standard. Within the window of Compound No 17 partial signal from ^{13}C -OCDF is observed next to the target (see also report from standard supplier Wellington)



Compound	Ret.-Time	Quantifier	Qualifier
1. 2378-TCDF	25.048	303.90>240.95	305.90>242.95
2.2378-TCDD	26.023	319.90>256.90	321.90>258.90
3. 12378-PeCDF	31.099	339.90>276.90	337.90>274.90
4. 23478-PeCDF	32.715	339.90>276.90	337.90>274.90
5. 12378-PeCDD	33.192	355.90>292.90	353.90>290.90
6. 123478-HxCDF	36.064	373.80>310.90	375.80>312.90
7. 123678-HxCDF	36.176	373.80>310.90	375.80>312.90
8. 234678-HxCDF	36.704	373.80>310.90	375.80>312.90
9. 123789-HxCDF	37.286	373.80>310.90	375.80>312.90
10.123478-HxCDD	36.862	389.80>326.90	391.80>328.80
11. 123678-HxCDD	36.942	389.80>326.90	391.80>328.80
12. 123789-HxCDD	37.106	389.80>326.90	391.80>328.80
13.1234678-HpCDF	38.312	407.80>344.80	409.80>346.80
14. 1234789-HpCDF	39.247	407.80>344.80	409.80>346.80
15. 1234678-HpCDD	38.994	423.80>360.80	425.80>362.80
16. OCDF	40.671	441.80>378.80	443.80>380.80
17. OCDD	40.591	457.70>394.70	459.70>396.70
18. 13C-2378-TCDF	25.027	315.95>251.95	317.95>253.95

Compound	Ret.-Time	Quantifier	Qualifier
19. 13C-1234-TCDD	25.245	331.90>268.00	333.90>270.00
20. 13C-2378-TCDD	25.994	331.90>268.00	333.90>270.00
21. 13C-12378-PeCDF	31.073	351.90>287.90	349.90>285.90
22. 13C-23478-PeCDF	32.703	351.90>287.90	349.90>285.90
23. 13C-12378-PeCDD	33.179	365.90>301.90	367.90>303.90
24. 13C-123478-HxCDF	36.055	385.80>321.90	387.80>323.90
25. 13C-123678-HxCDF	36.169	385.80>321.90	387.80>323.90
26. 13C-234678-HxCDF	36.692	385.80>321.90	387.80>323.90
27. 13C-123789-HxCDF	37.28	385.80>321.90	387.80>323.90
28. 13C-123478-HxCDD	36.856	399.90>335.90	401.80>337.90
29. 13C-123678-HxCDD	36.935	399.90>335.90	401.80>337.90
30. 13C-123789-HxCDD	37.099	399.90>335.90	401.80>337.90
31. 13C-1234678-HpCDF	38.308	419.80>355.90	421.80>357.90
32. 13C-1234789-HpCDF	39.242	419.80>355.90	421.80>357.90
33. 13C-1234678-HpCDD	38.99	435.80>371.80	437.80>373.80
34. 13C-OCDD	40.587	469.80>405.80	471.80>407.80
35. 13C-OCDF	40.667	453.80>389.80	455.80>391.80

Table 2: Compound table list with internal standards.

Real world samples

In figure 3 the results recorded with a fish oil sample are shown.

The concentrations calculated were TCDF 0.089, PeCDF 0.049, HxCDF 0.0714, 0.012, HpCDD 0.03, OCDF 0.024, OCDD 0.16 pg/2 μ l.

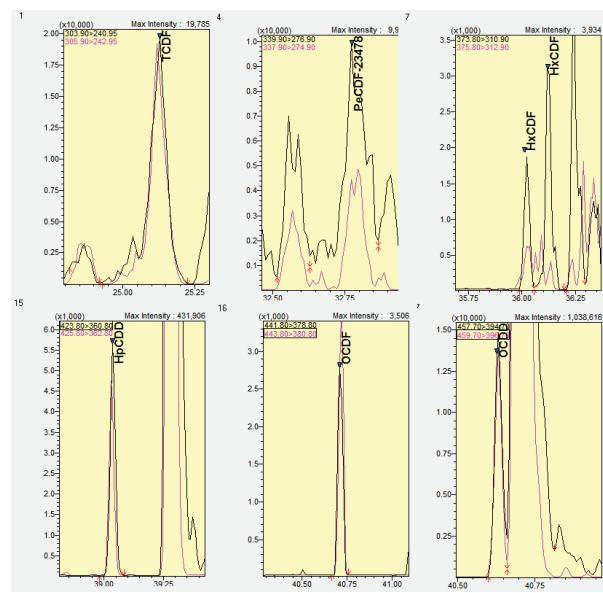
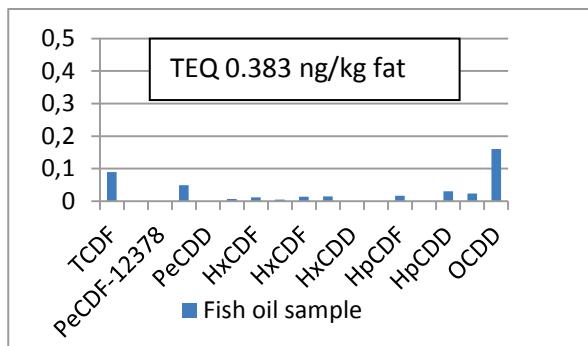


Figure 3: Results for detected compounds in a fish oil sample. Left: Concentration calculated for each congener. Right: peaks from the quantitation window

Each congener shows different toxicity which is expressed by the Toxic Equivalent Factor (TEF). This means that the analytical results relating to all the individual dioxin and dioxin-like PCB congeners of toxicological concern are expressed in terms of a quantity, namely the toxic equivalent (TEQ).

The Toxic Equivalent Factors are according to WHO from 2005 and are for the dibenzo-p-dioxins and furans: 2378-TCDD 1, 12378-Pe

CDD 1, 123478-HxCDD 0.1, 123678-HxCDD 0.1, 123789-HxCDD 0.1, 1234678-HpCDD 0.01, CDD 0.0003 and 2378-TCDF 0.1, 12378-Pe CDF 0.03, 123478-HxCDF 0.1, 123678-HxCDF 0.1, 123789-HxCDF 0.1, 1234678-HpCDF 0.001, 1234789-HpCDD 0.01, and OCDF 0.0003. The main contribution to the TEQ value of 0.383 ng/kg fat in figure 3 comes from TCDF (TEQ is calculated by multiplication of the concentration).

Comparison of results with HRMS

A total number of more than 50 samples were measured with both technologies. In figure 4, a component based comparison is shown for an animal feed and fish sample. The TEQ values calculated from these samples were for the

animal feed sample 0.0899 ng/kg (GCHRMS) and 0.0895 ng/kg (GCMSMS) and for the fish sample 0.307 ng/kg (GCHRMS) and 0.324 ng/kg (GCMSMS).

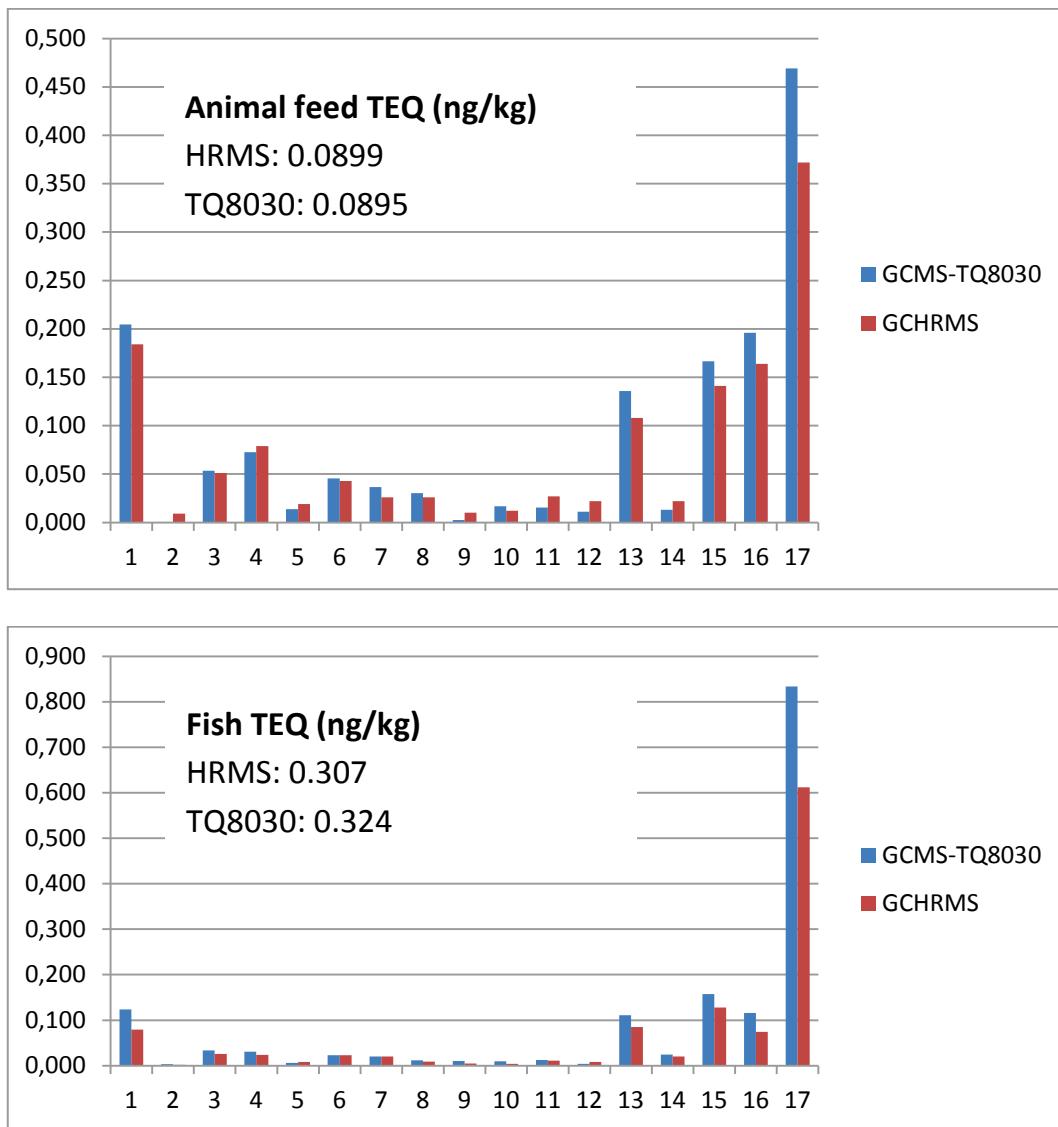


Figure 4: Comparison of concentrations (pg/ μ l) of individual PCDD and PCDF congeners determined with an animal feed (top) and fish sample (bottom). The x-axis numbers refer to the compounds listed in table 2.

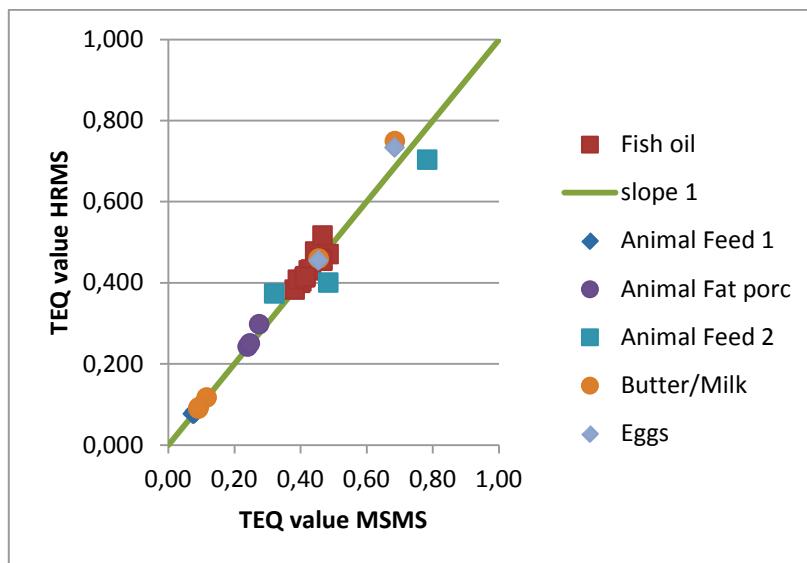


Figure 5: TEQ values (upper bound) in ng/kg calculated from GCHRMS and GCMSMS for various matrices

Then the method was applied to more than 50 samples. In figure 5 TEQ values calculated from GCHRMS and GCMSMS data are plotted against each other for various matrices. In addition the ideal curve with slope 1 is shown

as well. To have a better indication on the statistics, figure 6 shows the percentage deviation of 14 fish oil samples with TEQ values (upper bound) of 0.383 to 0.477 ng/kg fat.

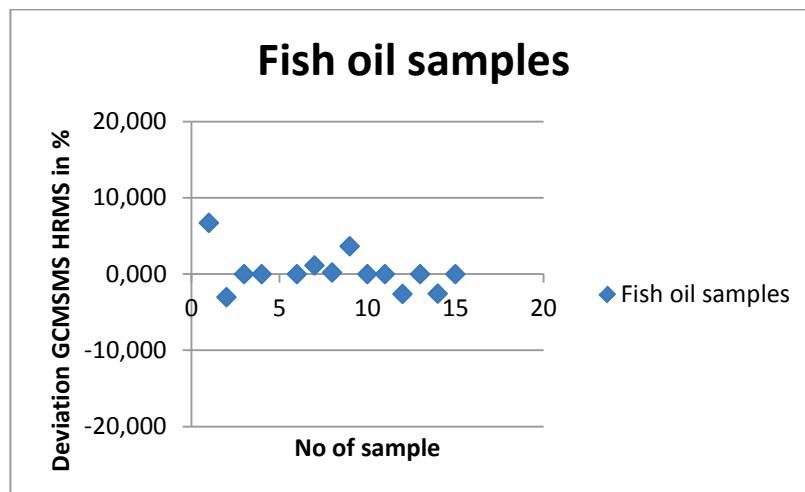


Figure 6: 14 samples (fish oil) are plotted to show the percentage deviation of the results obtained with GCMSMS relative to the ones obtained with GCHRMS.

Discussion

The TEQ values derived from the GCMSMS methods shown above, indicate a very good correlation with the established HRGCMS 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%.

Conclusions

The data shown in this application news indicate that the GCMS-TQ8030 proves sufficient accuracy for quantitative screening of dioxins in food and feed samples.

The maximum deviation of TEQ values calculated from GCMSMS data compared to the one from HRMS were below 10% for many matrices measured, even for low TEQ values below 0.5 ng/kg.

¹⁾ Sample preparation and measurement were performed at SGS Antwerp: HRMS: Waters Autospec, MSMS: Shimadzu GCMS-TQ8030

References

- [1] Commission Regulation (EC) No 882/2012 of 28 March 2012 amending Regulation (EC) No 152/2009 of (OJ L 91, 29.3.2012, p. 8–22)
- [2] Commission Regulation (EU) No 589/2014 of 03 June 2014 repealing Regulation (EU) No 252/2012
- [3] M. Geissler, S. Schröder Lab&more 2/11, p20
- [4] A. Kotz et al, Organohalogen Compounds Vol. 74, 156-159 (2012)

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