Determination of Polychlorinated Dibenzo-p-dioxins (PCDD) and Polychlorinated Dibenzofurans (PCDF) in Foodstuffs and Animal Feed using the Agilent 7000 Triple Quadrupole GC/MS System

Application Note

Food Safety

Authors

Prof. Dr. Peter Fürst
Dr. Thorsten Bernsmann
Dominik Baumeister
Chemical and Veterinary Analytical Institute
Münsterland-Emscher-Lippe
(Chemisches und Veterinär-untersuchungsamt, CVUA-MEL)
Joseph-König-Str. 40
48147 Münster
Germany

Chris Sandy
Agilent Technologies UK Ltd
610 Wharfedale Road
Winnersh Triangle
Wokingham
Berkshire
RG41 5TP
UK

Abstract

A method has been developed on the Agilent 7000 GC Triple Quadrupole GC/MS system for the analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in foodstuffs and animal feed. The method was shown to give linear response over the required concentration range, good repeatability of response and quantitation down to low pg TEQ/g levels.

Agilent Technologies

Introduction

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are highly toxic persistent organic pollutants (POP) with properties that are detrimental to human health and have been linked to causing cancer, endocrine disruption, and reproductive disorders. PCDD and PCDF are not manufactured deliberately but are the byproducts of the combustion of contaminated chemical waste, chemical and pesticide manufacturing, pulp and paper bleaching processes and other sources. PCDD and PCDF are lipophilic chemicals that accumulate in the fatty tissues of animals that form part of the human food chain. It is estimated that more than 80% of human exposure to dioxins derives from food of animal origin.

There have been several incidents of dioxin contamination in the human food chain over the past 20 years. One of the most recent was in December 2008 when contaminated pork and beef products were discovered in the Republic of Ireland [1] during routine testing.

Current legislation in the United States [2] and the European Union, [3,4] requires the confirmation and quantitation of dioxins, furans, and dioxin-like polychlorinated biphenyls (dl-PCBs) in foodstuffs and animal feed by isotope dilution capillary gas chromatography/ high resolution mass spectrometry (GC/HRMS). Additionally, EU Legislation does make provisions for the screening of dioxins in foodstuffs and animal feed by other mass spectrometric techniques or by bioassays. The specific compounds covered by the EU Legislation are shown in Table 1, along with the Toxic Equivalency Factors (TEF) relating the toxicity of each individual analyte to 2,3,7,8 tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which is assigned a TEF value of 1. The individual concentration of each dioxin, furan, and dl-PCB found in foodstuff and animal feed samples is multiplied with the respective TEF and after summation the total concentration is expressed as the Toxic Equivalent (TEQ) in terms of pg TEQ/g fat .

Table 1. PCDD, PCDF and dl-PCB congeners specified in EU Legislation along with the TEF values stipulated in 1998 and 2005. (WHO_{05} changes indicated in italics)

Compound	TEF WHO_{98}	TEF WHO_{05}	Compound	TEF WHO_{98}	TEF WHO_{05}
Chlorinated dibenzo-p-dioxins			Non-ortho substituted PCBs		
2378-TCDD	1	1	PCB-77	0.0001	0.0001
12378-PeCDD	1	1	PCB-81	0.0001	*0.0003*
123478-HxCDD	0.1	0.1	PCB-126	0.1	0.1
123678-HxCDD	0.1	0.1	PCB-169	0.01	*0.03*
123789-HxCDD	0.1	0.1			
1234678-HpCDD	0.01	0.01			
OCDD	0.0001	*0.0003*			
Chlorinated dibenzofurans			Mono-ortho substituted PCBs		
2378-TCDF	0.1	0.1	PCB-105	0.0001	*0.00003*
12378-PeCDF	0.05	*0.03*	PCB-114	0.0005	*0.00003*
23478-PeCDF	0.5	*0.3*	PCB-118	0.0001	*0.00003*
123478-HxCDF	0.1	0.1	PCB-123	0.0001	*0.00003*
123678-HxCDF	0.1	0.1	PCB-156	0.0005	*0.00003*
234678-HxCDF	0.1	0.1	PCB-157	0.0005	*0.00003*
123789-HxCDF	0.1	0.1	PCB-167	0.00001	*0.00003*
1234678-HpCDF	0.01	0.01	PCB-189	0.0001	*0.00003*
1234789-HpCDF	0.01	0.01			
OCDF	0.0001	*0.0003*			

The maximum levels for PCDD, PCDF, and dl-PCB in certain foodstuffs as prescribed by EU legislation are given in Table 2.

Table 2. Maximum Levels for PCDD, PCDF and Dioxin-like PCB in Certain Foodstuffs, as Specified in EU Regulation (EC) No 1881/2006

	Maximum levels	
Foodstuff	Sum of Dioxins (WHO-PCDD/F-TEQ)	Sum of Dioxins and dl-PCB (WHO-PCDD/F-PCB-TEQ)
Meat and meat products (excluding edible offal) of the following animals :		
Bovine animals and sheep	3.0 pg/g fat	4.5 pg/g fat
Poultry	2.0 pg/g fat	4.0 pg/g fat
Pigs	1.0 pg/g fat	1.5 pg/g fat
Raw milk and dairy products, including butter fat	3.0 pg/g fat	6.0 pg/g fat
Hens' eggs and egg products	3.0 pg/g fat	6.0 pg/g fat

This application note describes a sensitive and reproducible method that meets the requirements of EU Legislation for the screening of PCDD and PCDF in foodstuffs using the Agilent 7000 Triple Quadrupole GC/MS/MS system.

Experimental

Calibration Standards

Native PCDD and PCDF calibration mixtures and their ^{13}C-isotope labeled internal standards were obtained from Cambridge Isotope Laboratories and Wellington Laboratories Inc.

Sample Preparation and Analysis

The most frequently used methods for the determination of PCDD/PCDF and dl-PCB in foodstuffs and animal feed combine fat extraction (for example, Soxhlet or extraction with organic solvents) with cleanup steps using different column chromatographies, such as silica gel coated with sulphuric acid, florisil, alumina, and active carbon. The isotope labeled analogues of all PCDD/PCDF with 2,3,7,8-chlorine substitution were added at the beginning of the extraction. The extract was collected as three fractions containing dioxins (2), mono-ortho-PCB and indicator PCB (1a), and non-ortho PCBs (1b), by eluting with various solvents. After addition of a syringe spike ($^{13}C_{12}$ -1,2,3,4-TCDD), the extracts were evaporated under a gentle stream of nitrogen, reconstituted with toluene, and analyzed with GC/MS/MS. The dioxin fraction was reconstituted with 20 µL of toluene, the non-ortho PCB fraction in 40 µL of toluene and the mono-ortho and indicator PCB fraction in 250 µL of toluene.

A flow diagram summarizing the sample preparation steps is shown in Figure 1.

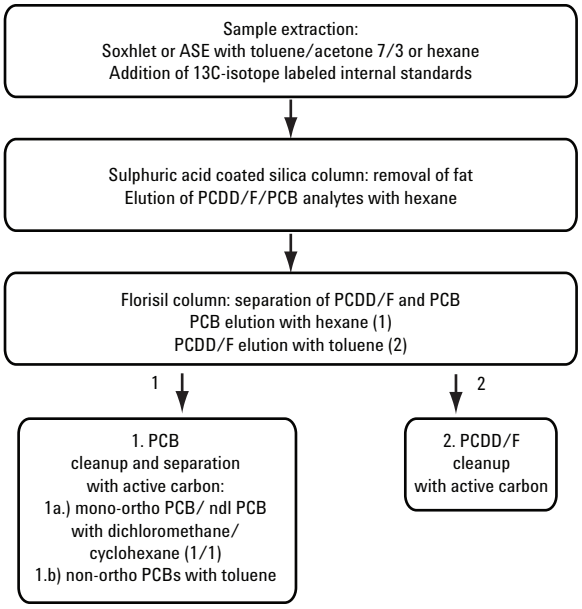

Figure 1. Flow diagram of the sample extraction and cleanup procedures.

The analysis was performed on an Agilent 7000 Triple Quadrupole GC/MS system with an Agilent 7890 GC. The 7890 GC was configured with a carbon dioxide cooled Multimode Inlet (MMI), a 2 m × 0.25 mm id uncoated deactivated capillary column linked to a pressure controlled tee (PCT) and an Agilent J&W DB-5ms UI 60 m × 0.25 mm, 0.25 µm capillary column. The chromatographic method was retention time locked (in direct connect mode) using PCB 105 to a retention time of 34.0 minutes.

The instrument conditions are listed in Table 3. A schematic diagram of the GC/MS/MS system is shown in Figure 2. The 7000 Triple Quadrupole GC/MS was operated in MS/MS-EI (electron ionization) Multiple Reaction Monitoring (MRM) mode. Each analyte and its associated ^{13}C-Internal standard was measured using two precursor ions and two different product ions. A full list of the analyte retention times and MRM settings are given in Table 4. The MRM settings consist of five time segments, each segment monitoring the tetra, penta, hexa, hepta, and octa dioxin and furan isomers, respectively. Dwell times were set to 75 ms for the native analytes and to 25 ms for all internal standards.

An Agilent 7693 Automatic Liquid Sampler with the sampler tray cooled to 5 °C was used to make 2-µL pulsed cold splitless injections using a 10-µL syringe.

Table 3. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Column (1)	2.0 m x 0.25 mm uncoated siltek deactivated fused silica
Pressure controlled tee	Agilent p/n G3186B
Column (2)	Agilent J&W DB-5ms UI 60 m × 0.25 mm, 0.25 µm (122-5562UI)
Back Flush time	15.0 minutes after injection
Back flush flow rate	Column (1) - 5.0 mL/min, concurrent back flush
Injection	2 µL cold pulsed splitless using CO_2 cooled Multi Mode Inlet (MMI)
Inlet temperature program	100 °C (0.05 min), 600 °C/min to 300 °C
Injection Pulse Pressure	30 psi until 1.0 min
Purge Flow to Split Vent	40 mL/min at 1.5 min
Carrier Gas	Helium, Column (1) constant flow 0.9 mL/min Helium, Column (2) constant flow 1.0 mL/min
RTL Compound	PCB 105, Locked RT = 34.0 minutes
Oven program	130 °C (2.0 min hold), 10 °C/min to 200 °C (16 min), 5 deg °C/min to 235 °C (7 min), 5 °C/min to 350 °C
MS Transfer line temp	300 °C
MS Conditions	
Tune	EI Autotune
Gain	100
MS1 Resolution	Wide
MS2 Resolution	Wide
Dwell Times	Natives 75 ms, Labeled compounds 25 ms
Collision Energies	Table 4
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
Solvent delay	25.0 minutes

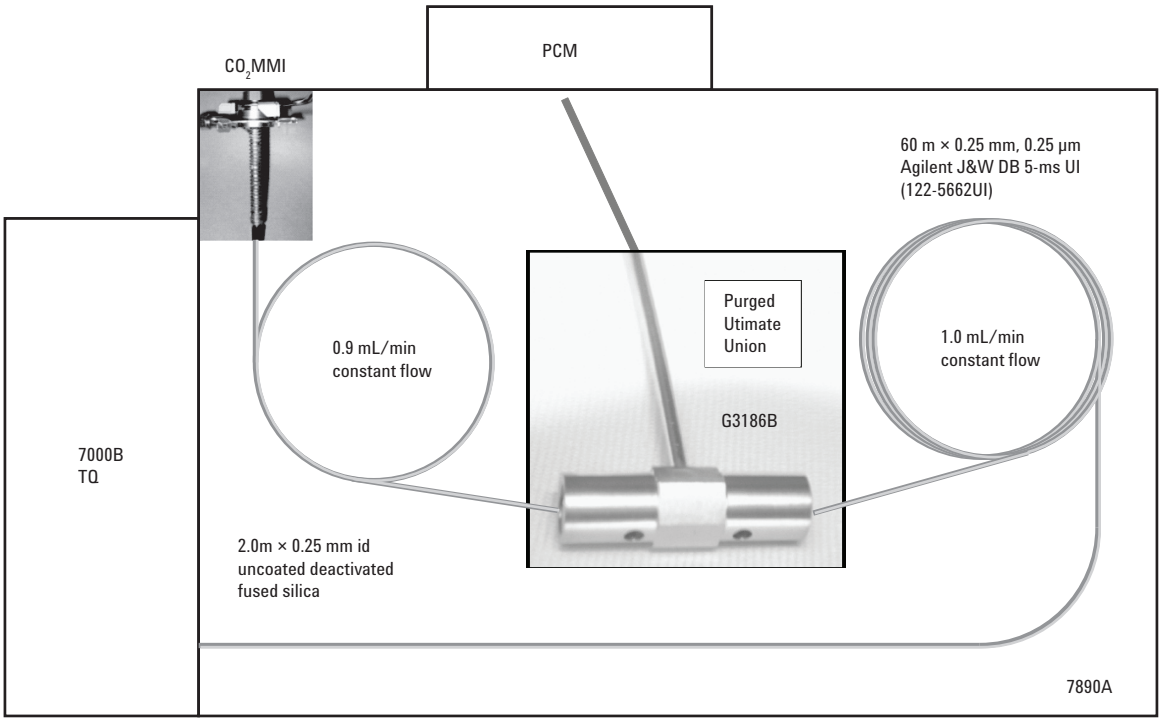

Figure 2. Schematic diagram of the GC/MS/MS hardware.

Table 4. MS/MS Settings for PCDD, PCDF and ^{13}C-Internal Standards

TS	Segment start time (min)	Peak number	Analyte	RT (min)	Quant precursor	Product	CE (V)	Qual precursor	Product	CE (V)
1	25.0									
		1	13C-2378-TCDF	35.43	315.9	251.9	33	317.9	253.9	33
		2	2378-TCDF	35.47	303.9	240.9	33	305.9	242.9	33
		3	13C-1234-TCDD	35.77	331.9	267.9	24	333.9	269.9	24
		4	13C-2378-TCDD	36.79	331.9	267.9	24	333.9	269.9	24
		5	2378-TCDD	36.80	319.9	256.9	24	321.9	258.9	24
2	40.0									
		6	13C-12378-PeCDF	42.55	351.9	287.9	35	349.9	285.9	35
		7	12378-PeCDF	42.56	339.9	276.9	35	337.9	274.9	35
		8	13C-23478-PeCDF	44.00	351.9	287.9	35	349.9	285.9	35
		9	23478-PeCDF	44.02	339.9	276.9	35	337.9	274.9	35
		10	13C-12378-PeCDD	44.45	365.9	301.9	25	367.9	303.9	25
		11	12378-PeCDD	44.48	355.9	292.9	25	353.9	290.9	25
3	46.0									
		12	13C-123478-HxCDF	48.04	385.8	321.9	35	387.8	323.9	35
		13	123478-HxCDF	48.06	373.8	310.9	35	375.8	312.9	35
		14	13C-123678-HxCDF	48.21	385.8	321.9	35	387.8	323.9	35
		15	123678-HxCDF	48.22	373.8	310.9	35	375.8	312.9	35
		16	13C-234678HxCDF	48.96	385.8	321.9	35	387.8	323.9	35
		17	234678-HxCDF	48.97	373.8	310.9	35	375.8	312.9	35
		18	13C-123478-HxCDD	49.17	403.8	339.8	25	401.8	337.9	25
		19	123478-HxCDD	49.19	389.8	326.9	25	391.8	328.8	25
		20	13C-123678-HxCDD	49.30	403.8	339.8	25	401.8	337.9	25
		21	123678-HxCDD	49.32	389.8	326.9	25	391.8	328.8	25
		22	13C-123789HxCDD	49.63	403.8	339.8	25	401.8	337.9	25
		23	123789-HxCDD	49.65	389.8	326.9	25	391.8	328.8	25
		24	13C-123789-HxCDF	50.04	385.8	321.9	35	387.8	323.9	35
		25	123789-HxCDF	50.06	373.8	310.9	35	375.8	312.9	35
4	51.0									
		26	13C-1234678-HpCDF	51.84	419.8	355.8	36	421.8	357.8	36
		27	1234678-HpCDF	51.86	409.8	346.8	36	407.8	344.8	36
		28	13C-1234678-HpCDD	53.11	437.8	373.8	25	435.8	371.8	25
		29	1234678-HpCDD	53.13	423.8	360.8	25	425.8	362.8	25
		30	13C-1234789-HpCDF	53.69	419.8	355.8	36	421.8	357.8	36
		31	1234789-HpCDF	53.70	407.8	344.8	36	409.8	346.8	36
5	55.0									
		32	13C-OCDD	56.23	469.7	405.8	26	471.7	407.8	26
		33	OCDD	56.24	457.7	394.8	26	459.7	396.8	26
		34	13C-OCDF	56.41	453.7	389.8	35	455.7	391.8	35
		35	OCDF	56.42	441.7	378.8	35	443.7	380.8	35

Capillary flow technology and backflushing have proven to be invaluable tools in improving method robustness and chromatographic integrity for GC/MS analysis of samples with high matrix content [5]. Backflushing removes high-boiling matrix components from the system that would otherwise remain behind from injection to injection, causing retention time shifts, loss of chromatographic peak shapes, and eventual contamination of the mass spectrometer ion source.

The 2-m precolumn and pressure controlled tee (PCT) were used to provide concurrent backflushing of the precolumn during the chromatographic run. Concurrent backflushing is a technique that works well in methods employing long (60 m) capillary columns that cannot be efficiently backflushed in postrun mode using a post-column connection to the PCT. The flow rate in the precolumn is reversed once all the analytes of interest have moved in to the 60-m analytical column. This is implemented by automatically reducing the pressure at the MMI 15 minutes after the sample injection takes place, which was determined experimentally by a sequence of standard injections with varying backflush times.

Results and Discussion

Chromatography

The chromatographic separation of the native PCDD and PCDF congeners is shown in Figure 3. The peak numbers refer to the entries in Table 4. The chromatographic run time for each sample was 60 minutes.

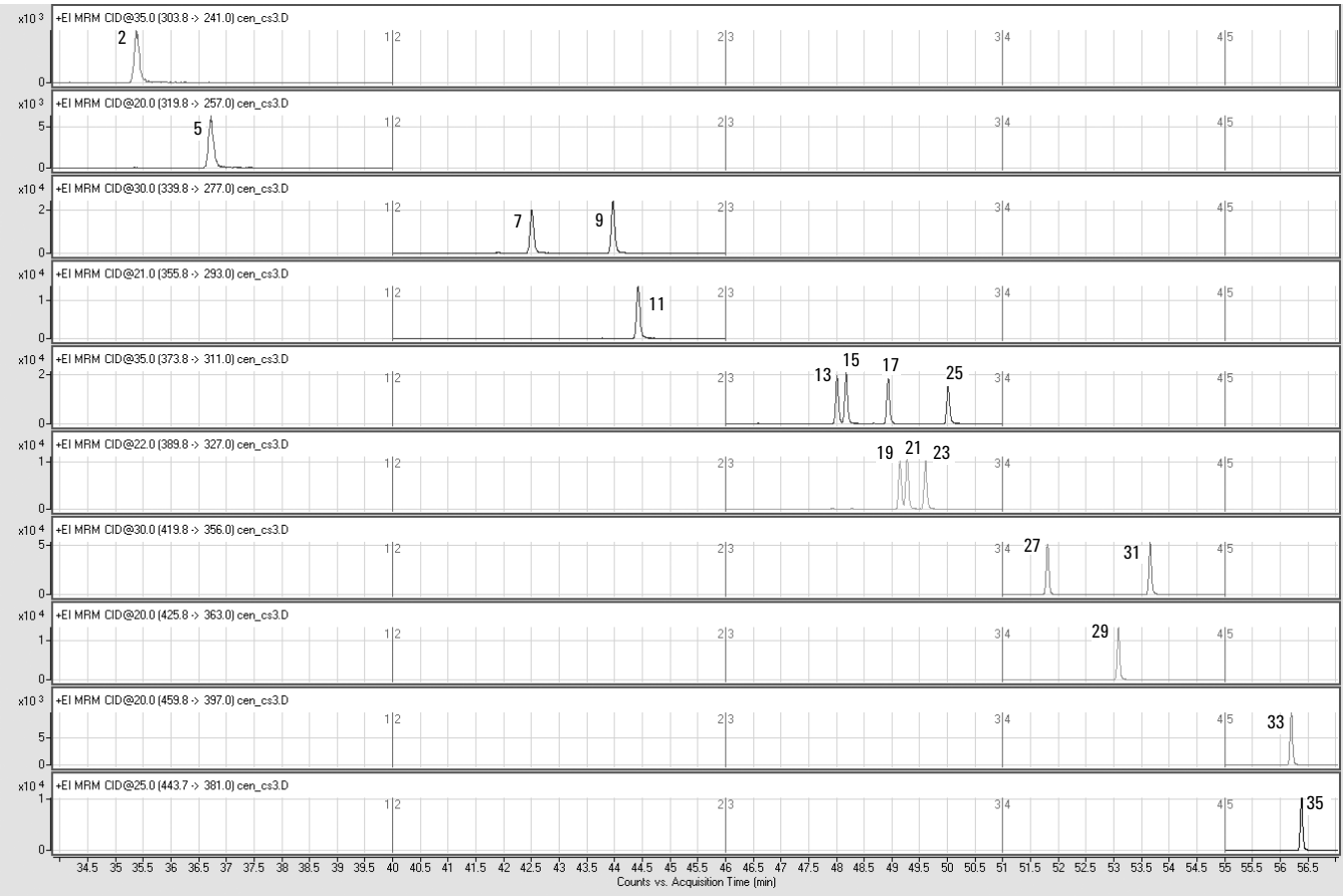

Figure 3. MRM chromatograms of native PCDD and PCDF congeners. (Peak numbers refer to analytes listed in Table 4).

Linearity of Response and Sensitivity

The PCDD and PCDF were measured using ^{13}C-labeled internal standard (ISTD) calibration. The seven-point ISTD calibration curves for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD are shown in Figures 4 and 5, respectively. Excellent linearity is shown for 2 µL injections of the calibration standards over the concentration range of 0.05 pg/µL to 5 pg/µL with R^2 values > 0.999. The insets in Figures 4 and 5 show the R^2 values for the average of response factors for these two dioxin congeners.

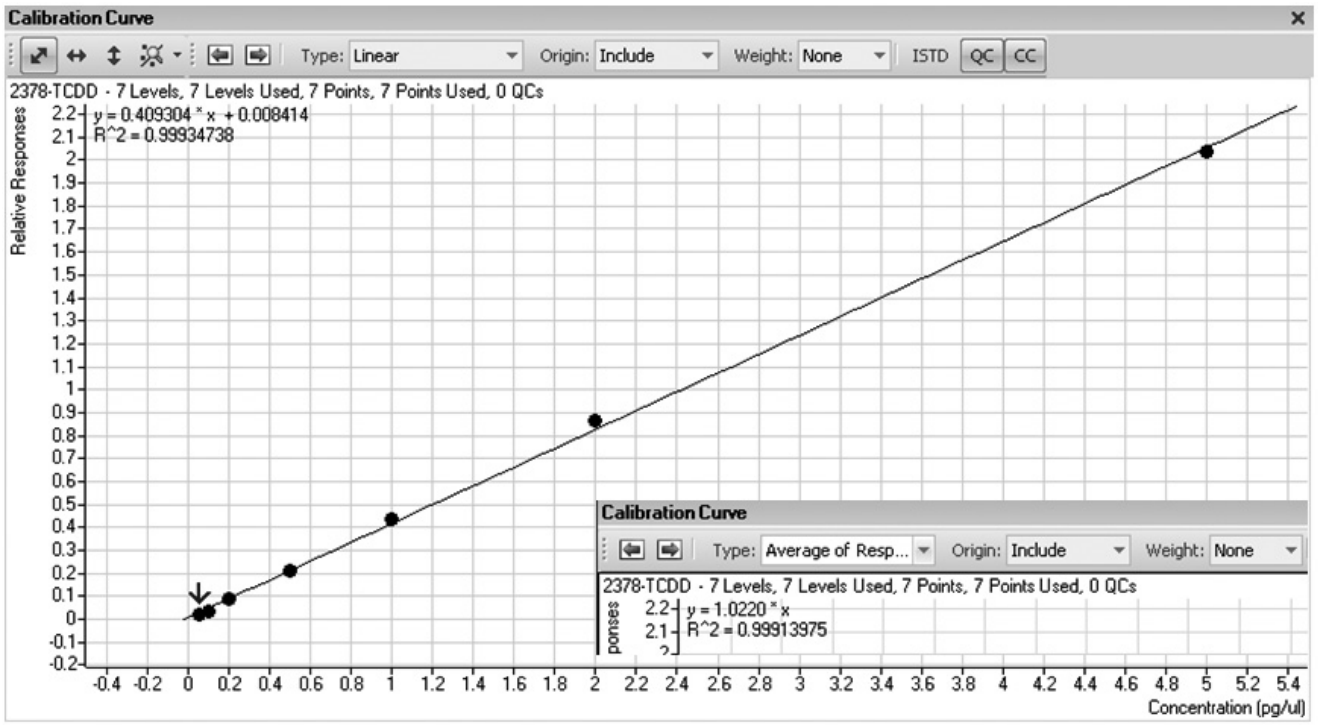

Figure 4. Calibration curve for 2,3,7,8-TCDD with both linear fit and average of response factors (inset).

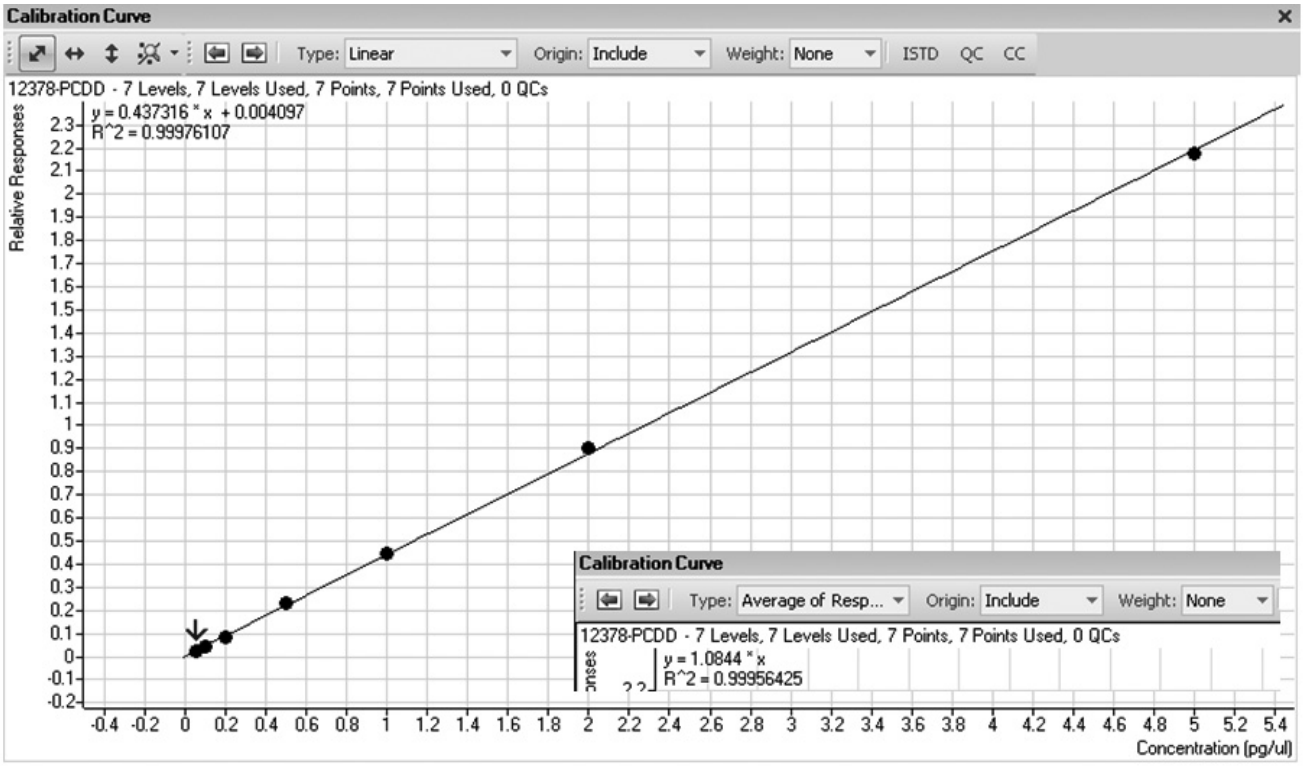

Figure 5. Calibration curve for 1,2,3,7,8-PeCDD with both linear fit and average of response factors (inset).

The linear calibration curve fits for all 17 PCDD and PCDF congeners are shown in Table 5.

*Table 5. Linear Correlation Coefficients for Seven-Point ISTD Calibration Curves over the Range 100 fg – 10 pg Injected. * (OCDD 500 fg – 50 pg injected)*

Analyte	R^2	Analyte	R^2
2378-TCDD	0.99934	2378-TCDF	0.99984
12378-PeCDD	0.99976	12378-PeCDF	0.99909
123478-HxCDD	0.99994	23478-PeCDF	0.99995
123678-HxCDD	0.99905	123478-HxCDF	0.99971
123789-HxCDD	0.99977	123678-HxCDF	0.99983
1234678-HpCDD	0.99945	234678-HxCDF	0.99953
OCDD*	0.99780	123789-HxCDF	0.99972
		1234678-HpCDF	0.99971
		1234789-HpCDF	0.99991
		OCDF	0.99907

The selected reaction monitoring (MRM) chromatograms for the native PCDD and PCDF congeners for the lowest calibration standard (0.1 pg on-column) are shown in Figure 6.

Peak Area Precision and Peak Area Ratio Precision

The peak area precision (raw peak area) for the native PCDD and PCDF congeners was determined by spiking a pork fat extract with native PCDD and PCDF at a concentration of 100 fg/µL and ^{13}C-ISTD at 1pg/µL, respectively. A sequence of replicate 2-µL cold pulsed splitless injections (n = 15) was made. The %RSD values for the peak areas of native PCDD/PCDF and ^{13}C-ISTD are shown in Figure 7. All native congeners gave precision values less than 10% except for 1,2,3,4,6,7,8-HpCDD, which gave a value of 11.9 %. This slightly higher result may be attributed to the somewhat lower absolute response of this particular analyte. The ^{13}C-ISTD gave %RSD values of 5% or lower.

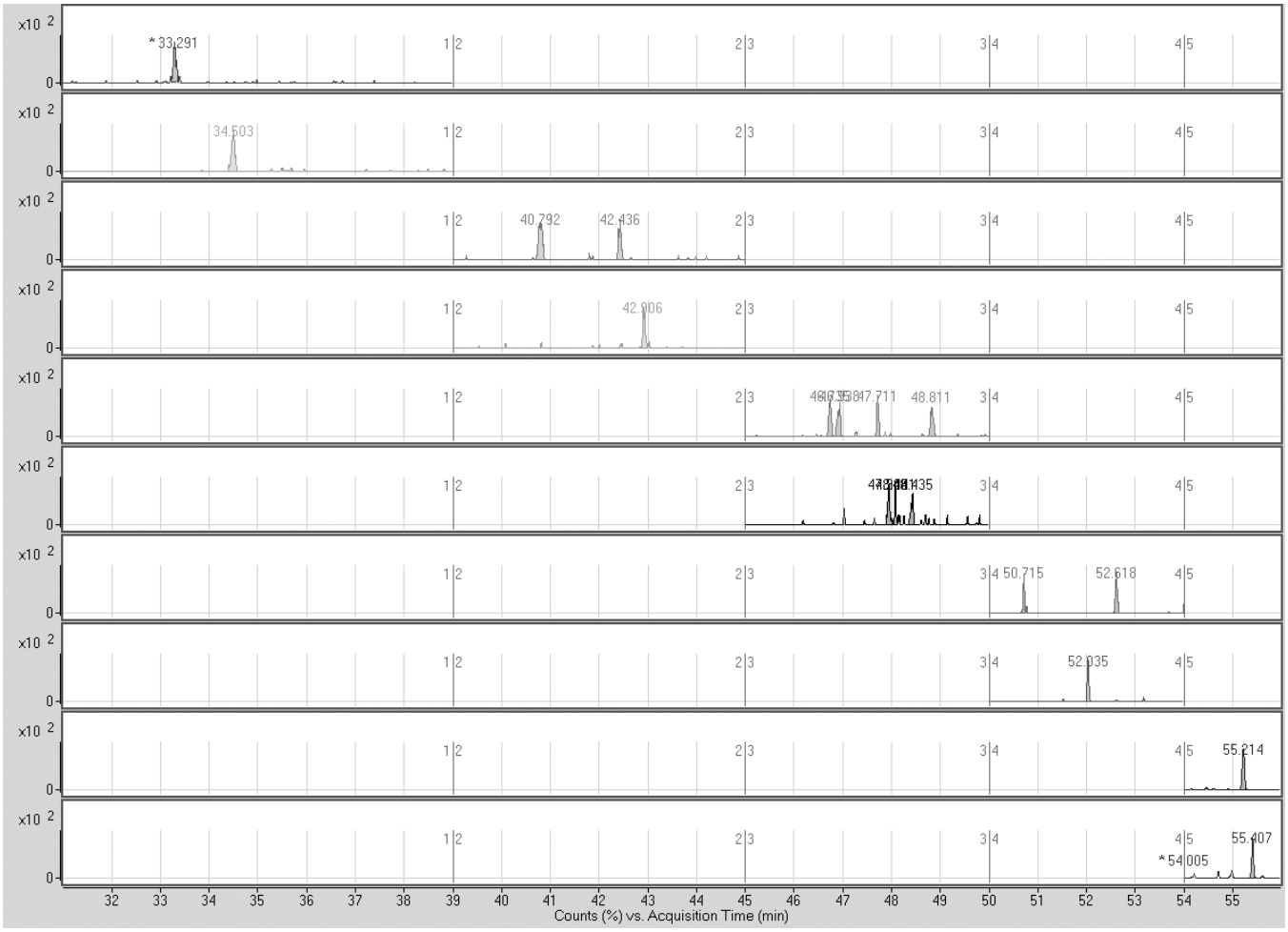

Figure 6. MRM chromatograms of native PCDD and PCDF congeners. Lowest calibration standard, 100 fg injected on-column (OCDD 500 fg injected on-column).

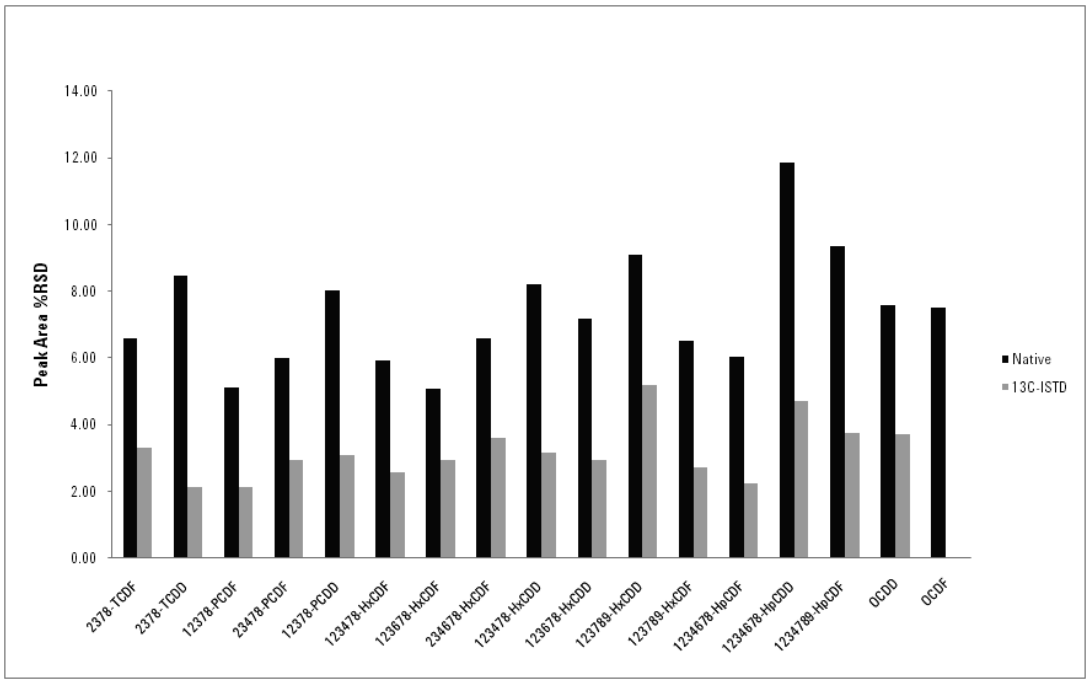

Figure 7. Repeatability of peak areas for native PCDD and PCDF congeners and ^{13}C-ISTD (n=15).

The peak area ratio precision (analyte peak area divided by its ^{13}C-ISTD peak area) was also determined for the 15 replicate injections. The %RSD values for the ratio of peak areas are shown in Figure 8. All analytes gave precision values less than 10% except for 1,2,3,4,6,7,8-HpCDD, which gave a %RSD value of 13.6 %.

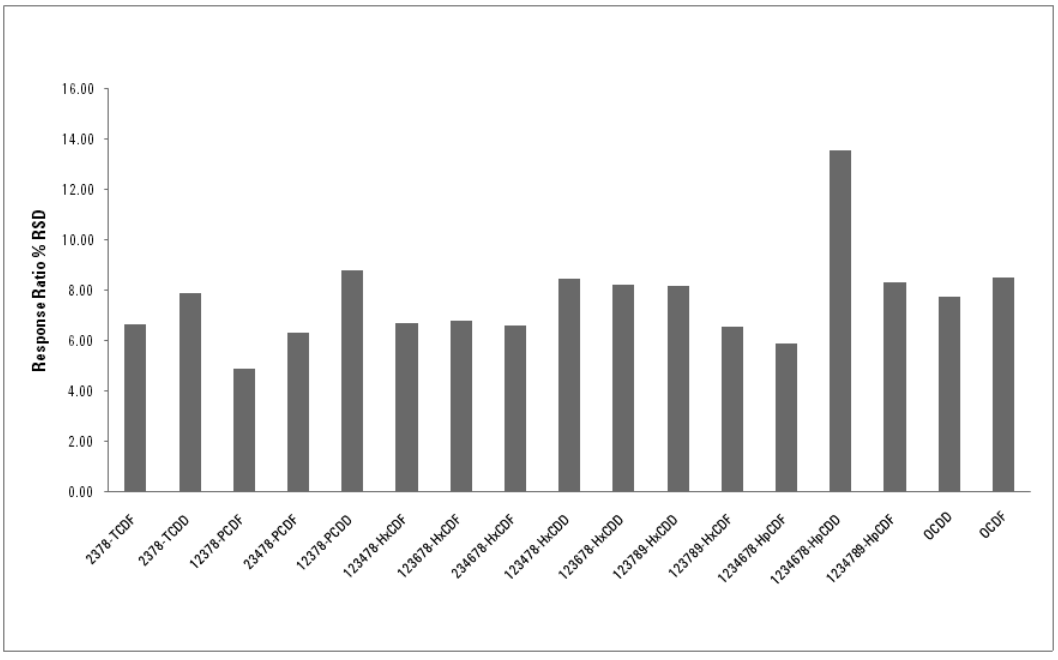

Figure 8. Repeatability of response ratios for native PCDD and PCDF congeners (n=15).

Sample Analysis

The MRM chromatograms for the tetra- and penta-CDF isomers present in a hen's egg extract are shown in Figure 9. The concentrations of the 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF were determined as 15.5, 3.4, and 3.1 pg/g fat, respectively.

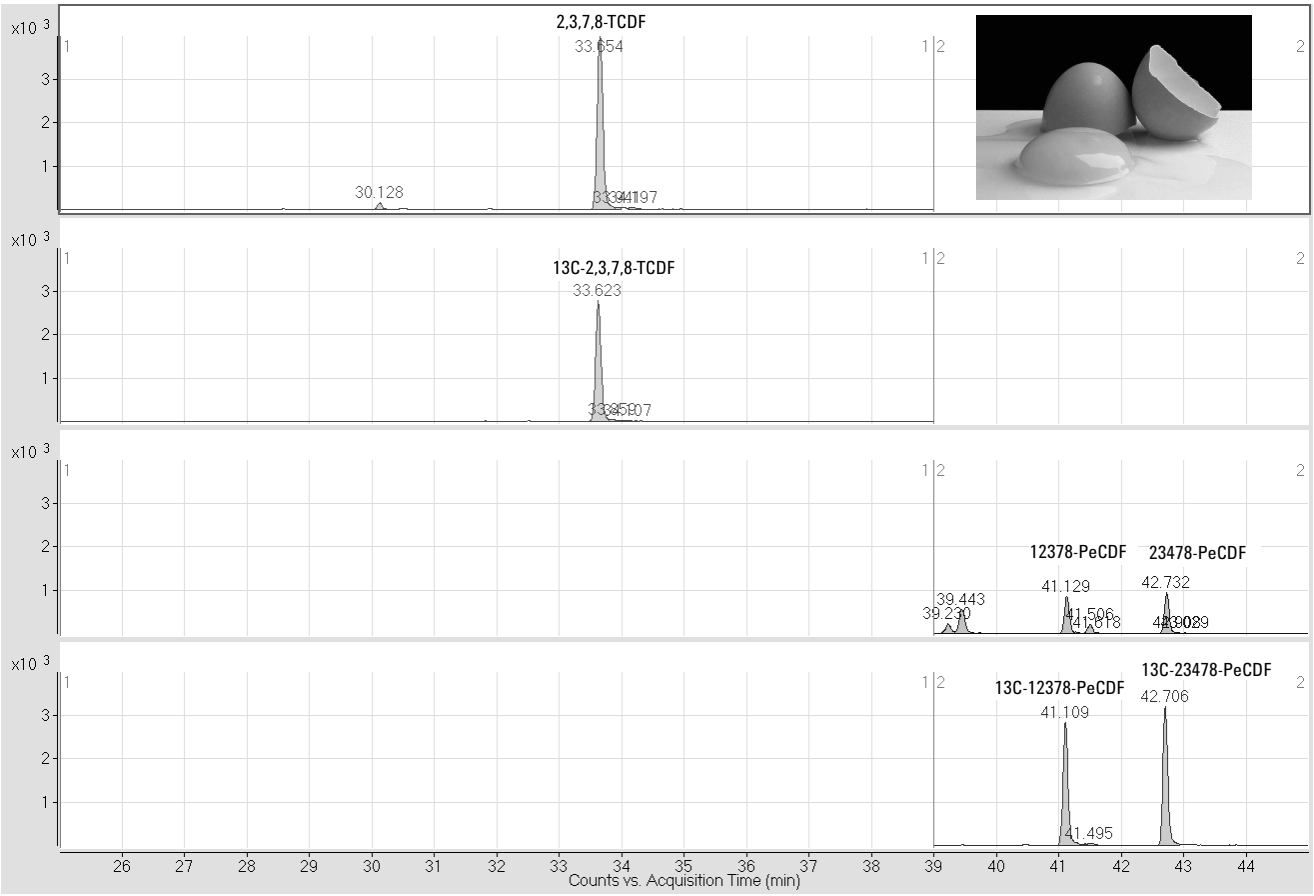

Figure 9. MRM chromatograms of tetra- and penta-CDF congeners and ^{13}C-ISTDs from a hen's egg extract.

An advantage of screening for dioxins and furans in foodstuffs and animal feed by GC/MS/MS, as opposed to using bio-assay, is that each congener is individually quantified. This allows the quantitative contribution of each PCDD and PCDF congener within the sample to be plotted. This, in turn, may provide a valuable clue as to the likely source of the contamination. The quantitative distribution of PCDD and PCDF congeners in a hen's egg extract is shown in Figure 10.

Samples of five different foodstuffs: liver (n=5), beef (n=4), poultry meat (n=6), hens' eggs (n=5), and animal feed (n=31) were extracted and analyzed using a GC High Resolution Mass Spectrometer (GC/HRMS) at a resolution of R=10,000. The same sample vials were then transferred to the Agilent 7000 GC/MS/MS system and reanalyzed.

Figure 11 shows the comparative sample results (upperbound values) of the two sets of measurements expressed as the percentage difference between the results obtained by the GC/HRMS and GC/MS/MS analyses.

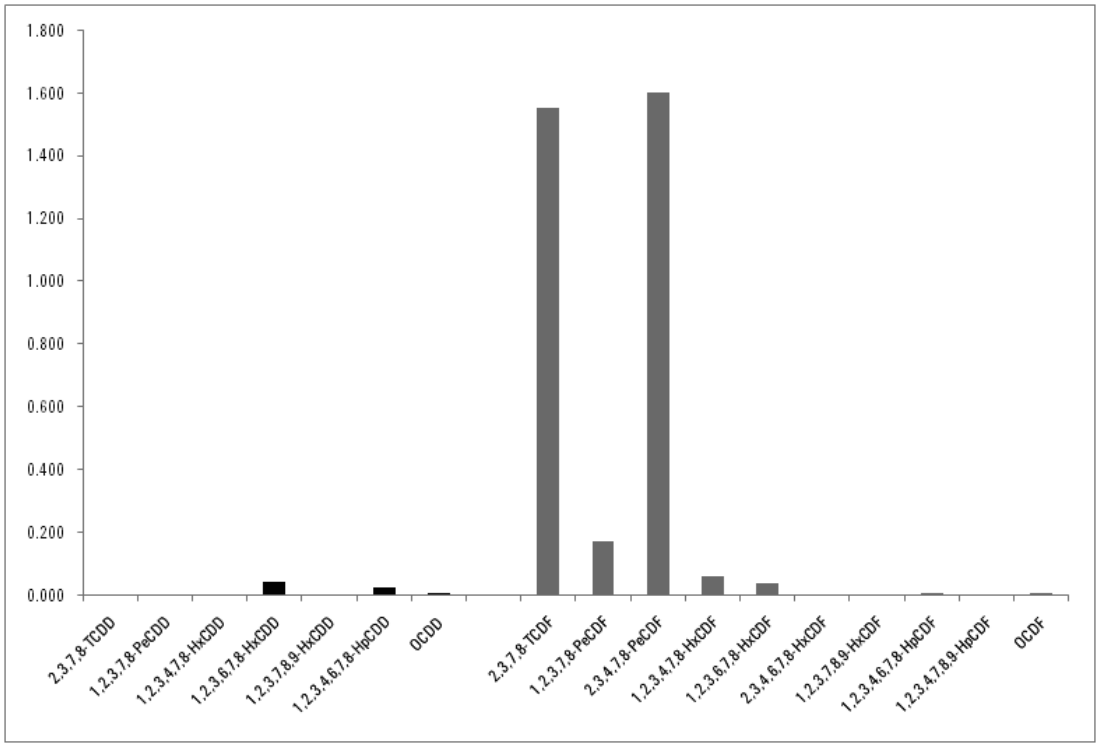

Figure 10. Quantitative distribution of PCDD and PCDF congeners in a hen's egg extract, units are pg TEQ/g fat.

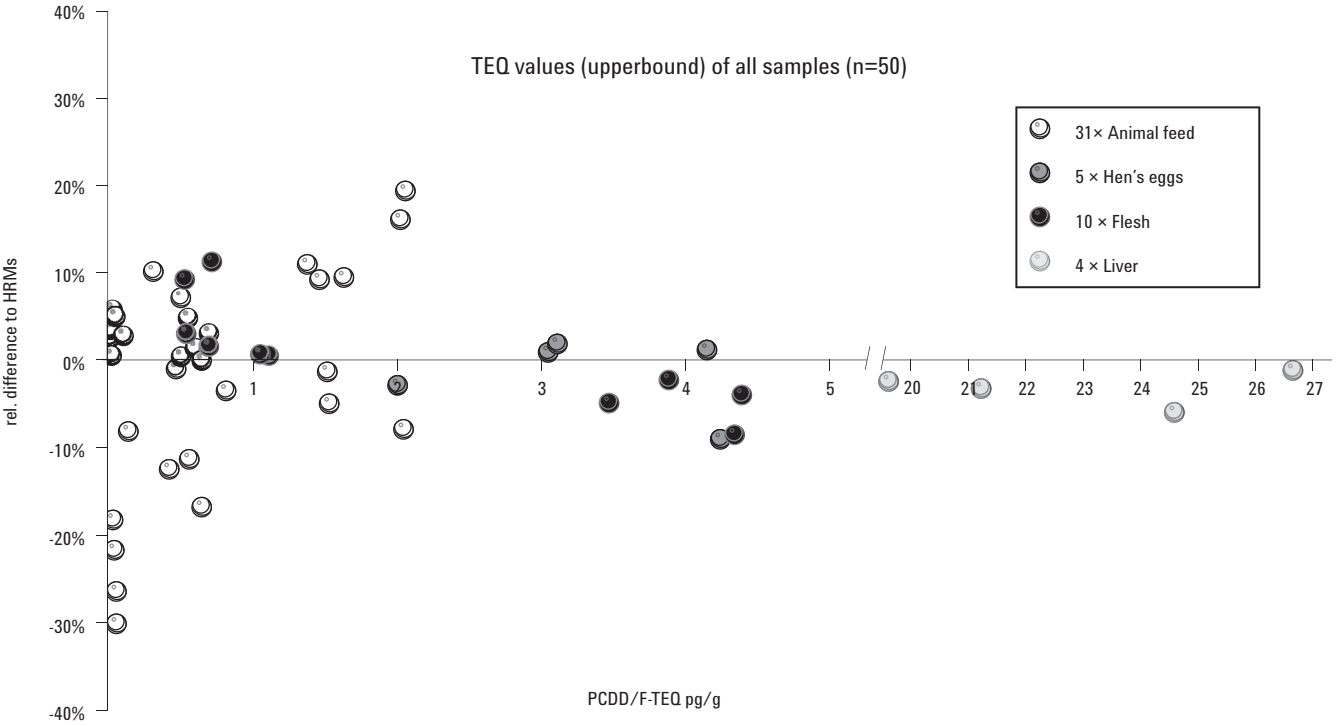

Figure 11. Comparative results (upperbound concentration values) for 50 food and feed samples analyzed by GC/HRMS and GC/MS/MS.

Figure 12 shows the comparative sample results (upperbound concentration values) of the two sets of measurements for those samples that gave values less than 3 pg TEQ/g. Additionally, Figure 12 is annotated with the Maximum Levels (ML) and Action Levels (AL) for poultry meat, hens' eggs, and animal feedstuff as prescribed by European Union Legislation.

Foodstuff samples that exhibited levels of total PCDD and PCDF congeners at upperbound values greater than 3 pg TEQ/g gave quantitative results by GC/MS/MS that were within ± 10% of the value obtained by GC/HRMS.

The agreement between the results obtained on the GC-HRMS and the GC/MS/MS for foodstuff and feedstuff samples at levels between 0.5 and 3 pg/g TEQ were within the range of ± 10 to ± 20%.

Only those animal feedstuff samples with results of 0.1–0.2 pg TEQ/g (well below the EU action level of 0.5 pg TEQ/g) gave result differences > 20% between the GC/HRMS and GC/MS/MS. This greater differential may be attributed to the results being expressed as the upperbound values and the lower limit of detection (LOD) achievable by the GC/HRMS system. In Animal Feedstuff samples, the GC/HRMS gave a range of LODs for the PCDD and PCDF congeners between 0.01–0.06 pg/g, whereas the GC/MS/MS gave 0.02–0.08 pg/g.

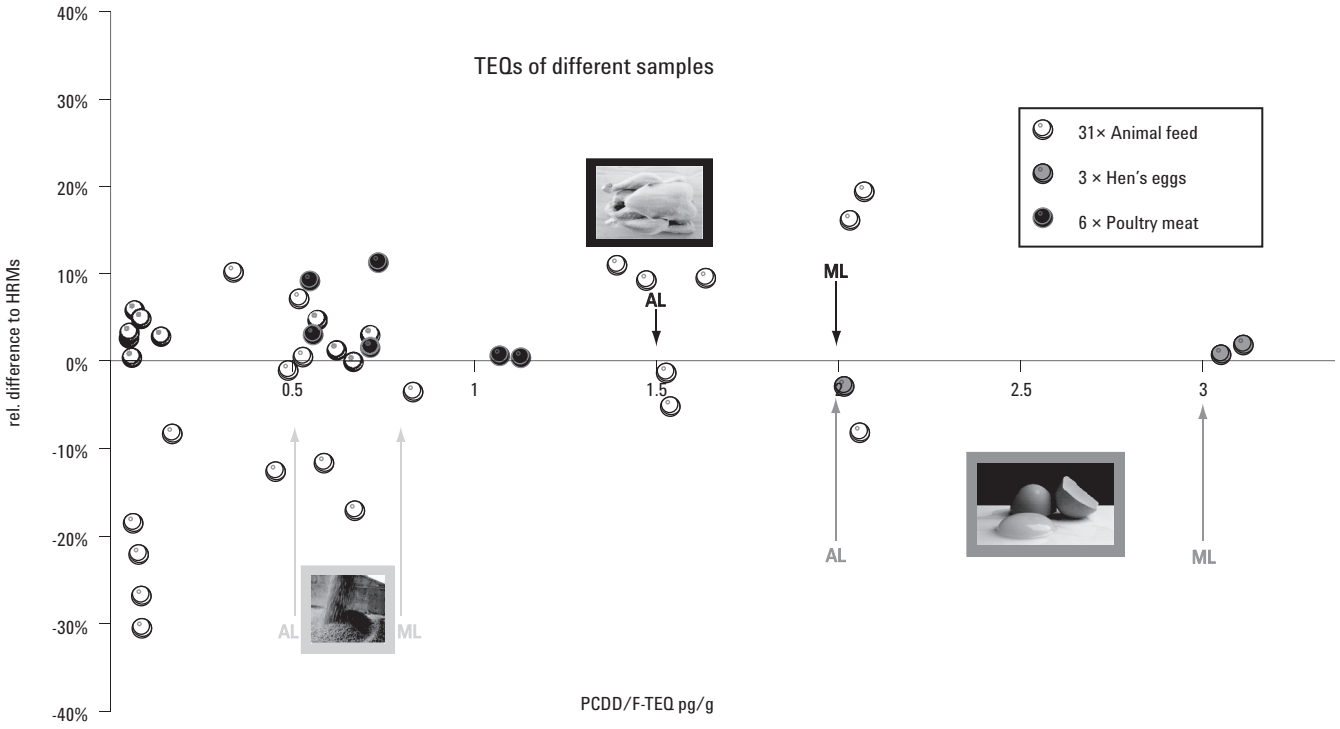

Figure 12. Comparative results (upperbound concentration values) for 40 food and feed samples analyzed by GC/HRMS and GC/MS/MS that gave values less than ~3 pg TEQ/g. ML= EU Maximum Level, AL = EU Action Level.

Conclusion

The Agilent 7000 Triple Quadrupole GC/MS system provides linear, reproducible and sensitive detection of PCDD and PCDF congeners in foodstuffs and animal feed samples down to low pg TEQ/g values. Comparison of analytical results of foodstuff and animal feed extracts by GC/HRMS and GC/MS/MS indicates the suitability of the Agilent 7000 Triple Quadrupole GC/MS system for routine screening of PCDD and PCDF congeners in foodstuffs and feedstuffs that meets the requirements of European Union legislation.

References

1. UK Food Standards Agency Ref 74/2008 - Emerging incident involving presence of dioxins in Irish pork meat.
2. US EPA Method 1613B - Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope dilution HRGC/HRMS.
3. Commission Regulation (EC) No 1883/2006 of 19 December 2006 Laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs.
4. Commission Regulation (EC) No. 152/2009 Annexe V letter B, 27 January 2009, "Laying Down the Methods of Sampling and Analysis for the Official Control of Feed".
5. C. Sandy, "Improving GC-MS Method Robustness and Cycle Times using Capillary Flow Technology and Back Flushing," Agilent Technologies publication 5990-3367EN (2009).

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

www.agilent.com/chem

Printed in the USA
November 15, 2010
5990-6594EN

Agilent Technologies