Validation of a Confirmatory GC/MS/MS Method for Dioxins and Dioxin-like PCBS to Meet the Requirements of EU Regulation 709/2014

Application Note

Food

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Abstract

Using the Agilent 7890B GC and the Agilent 7000C Series Triple Quadrupole GC/MS System, a GC/MS/MS method has been developed and fully validated to meet the requirements of EU Regulation 709/2014 for the monitoring of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in animal feedstuffs. It provides similar performance to GC/HRMS, the analytical platform required by previous EU regulations, in spite of the difference in mass analyzer technologies.

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are highly toxic Persistent Organic Pollutants (POPs). As such, they were regulated after the Stockholm convention for POPs in 2001 to safeguard the environment and human health [1]. Many of these compounds have been linked to cancer, endocrine disruption, and reproductive disorders. They are created as byproducts of industrial processes, pesticide manufacturing, combustion processes, and other sources.

These toxic compounds are very stable in the environment, and their lipophilic nature allows them to accumulate in the fat tissues of animals. Therefore, the European Commission requires that any food or animal feedstuffs released on the market must be monitored to not exceed assigned maximum levels (MLs) for these pollutants. European regulations also require enforcement of continuous food and feed monitoring of these compounds. These regulations enable efficient reduction in human exposure over time, and decreased daily human intake of these toxic compounds [1].

Historically, high resolution mass spectrometry (HRMS) was needed to confirm and quantify trace levels of dioxins. However, as of June 2014, the European Union (EU) has instituted regulation (709/2014) governing the levels of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs in food and feed that enables the use of gas chromatography/tandem mass spectrometry (GC/MS/MS) systems in confirmatory testing for compliance with EU MLs. This change was due to the realization that triple quadrupole mass spectrometers could provide performance similar to that seen with HRMS systems [2].

This application note describes a published study that validated the use of GC/triple quadrupole MS for the confirmatory analysis of dioxins and dioxin-like PCBs in vegetable oil [1]. Using the Agilent 7890GC and Agilent 7000 Triple Quadrupole GC/MS System, a method was validated that met the strict requirements for analytical criteria (for example, selectivity, accuracy, and reproducibility) set by the EU regulation. Results were similar to those that can be attained with GC/HRMS, thus providing a viable and economical alternative to the GC/HRMS approach.

Experimental

Reagents and standards

Solvents and reagents were obtained as described [1]. Quantitation of all congeners of PCDD/Fs (2,3,7,8-substituted) and non-ortho (NO-)PCBs (PCBs 81, 77, 126, and 169) was carried out using the corresponding ^{13}C-labeled internal standards (EDF-4144, Cambridge Isotope Laboratories (CIL)). Recovery standards (EDF-4145, syringe standard, CIL) were used for determination of recoveries. Calibration curve standards were also purchased from CIL for PCDD/Fs and NO-PCBs (EDF-4143). Internal standard spiking solution (MBP-MKX) of ^{13}C-labeled mono-ortho (MO-)PCBs (including PCBs 105, 114, 118, 123, 156, 157, 167, and 189) was obtained from Wellington Laboratories. The EC-4987, EC-5179, EC-4058 (CIL), and MBP-MKX standards were used to construct the calibration curve for MO-PCBs and NDL-PCBs (PCBs 28, 52, 101, 138, 153, and 180).

Instruments

This study was performed using an Agilent 7890B GC system coupled to an Agilent 7000C Series Triple Quadrupole GC/MS System. The instrument conditions are listed in Table 1. Every 10 days, calibration and tune of the instrument were repeated using the EI high sensitivity autotune mode, and instrument performance was verified.

Table 1. GC/MS Run Conditions

GC Conditions	
Column	Agilent DB-5 MS UI 60 m × 250 µm × 0.25 µm (p/n 122-5562UI) inlet PTV, outlet vacuum
Injection volume	PCDD/Fs and NO-PCBs: 5 µL MO and NDL-PCBs: 2 µL
Injection port	PTV cooled with liquid CO2
Injection port liner	Multibaffle, deactivated, PTV liner (p/n 5183-2037)
Injection mode	Solvent vent 45 °C (3 minutes), ramp at 720 °C/min to 320 °C Vent flow 100 mL/min pressure of 10 psi for 2.8 minutes Purge flow was set to 1200 mL/min after 5 minutes.
Carrier gas	Helium
Carrier gas mode	Constant flow
Column flow	0.96 mL/min
Retention time locking	PCB-105 locked to 19.66 minutes
Oven program	120 °C (5 minutes) 25 °C/min to 250 °C (5 minutes) 3 °C/min to 285 °C (15 minutes) The same program was used for the MO-PCB fraction, with the exception of 0 minutes hold at 285 °C
Total run time	41.6 minutes
MS Conditions	
Operation mode	Electron ionization (EI), Multiple Reaction Monitoring (MRM)
Transfer line temperature	280 °C
Source temperature	280 °C
Quadrupole temperature	150 °C

Multiple Reaction Monitoring (MRM) mode was used for data acquisition, with acquisition windows and dwell times adjusted to optimize acquisition frequency to obtain 10 data points for each peak. For each target, two MRM transitions were used, one for quantitation and one for qualification. The two transitions used two different and specific precursor ions (usually 2 Da offset) and two distinct product ions. Table 2 gives a full list of the analyte retention times and MRM transitions.

Quantitation was performed with the quantitative transition only, while the qualitative transition was used to verify the ion ratio between the two transitions. This procedure limited the risk of integrating wrong peaks or interferences. If the ratio did not fall between acceptable limits for Regulation 709/2014 (±15%), the chromatogram was inspected to ensure that the appropriate peaks were being integrated. This approach minimized the risk of integrating interferences or the wrong peaks. Retention time locking was employed, using PCB-105.

Sample preparation

Preparation of vegetable oil samples was performed as described [1].

Data acquisition and analysis

The data were acquired with Agilent MassHunter Acquisition Software (B.07.02). Data analysis was performed with Agilent MassHunter Quantitative Analysis Software (B.07.01).

Table 2. Acquisition Parameters for Native PCDD, PCDF Mono-Ortho, Non-ortho and NDL-PCB Congeners, and ^{13}C-Internal Standards

			Quantifier			Qualifier		
Name	**Type**	**RT***	**Precursor ion**	**Product ion**	**CE†(V)**	**Precursor ion**	**Product ion**	**CE†(V)**
^{13}C-PCB 28	ndl-PCB	14.19	268.0	198.0	26	270.0	200.0	26
PCB 28	ndl-PCB	14.19	256.0	186.0	26	258.0	188.0	26
^{13}C-PCB 52	ndl-PCB	14.79	301.9	231.9	28	303.9	233.9	28
PCB 52	ndl-PCB	14.79	289.9	219.9	28	291.9	221.9	28
^{13}C-PCB 101	ndl-PCB	16.81	337.9	267.9	28	339.9	269.9	28
PCB 101	ndl-PCB	16.81	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 123	MO-PCB	18.62	337.9	267.9	28	339.9	269.9	28
PCB 123	MO-PCB	18.62	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 118	MO-PCB	18.74	337.9	267.9	28	339.9	269.9	28
PCB 118	MO-PCB	18.74	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 114	MO-PCB	19.12	337.9	267.9	28	339.9	269.9	28
PCB 114	MO-PCB	19.12	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 153	ndl-PCB	19.43	371.9	301.9	28	373.9	303.8	28
PCB 153	ndl-PCB	19.43	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 105	MO-PCB	19.66	337.9	267.9	28	339.9	269.9	28
PCB 105	MO-PCB	19.66	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 138	ndl-PCB	20.46	371.9	301.9	28	373.9	303.8	28
PCB 138	ndl-PCB	20.46	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 167	MO-PCB	21.56	371.9	301.9	28	373.9	303.8	28
PCB 167	MO-PCB	21.56	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 156	MO-PCB	22.51	371.9	301.9	28	373.9	303.8	28
PCB 156	MO-PCB	22.51	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 157	MO-PCB	22.71	371.9	301.9	28	373.9	303.8	28
PCB 157	MO-PCB	22.71	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 180	ndl-PCB	23.14	405.8	335.8	30	407.8	337.8	30
PCB 180	ndl-PCB	23.14	393.8	323.8	30	395.8	325.8	30
^{13}C-PCB 189	MO-PCB	25.76	405.8	335.8	30	407.8	337.8	30
PCB 189	MO-PCB	25.76	393.8	323.8	30	395.8	325.8	30
^{13}C-PCB 80	non-Ortho PCB	16.23	301.9	231.9	28	303.9	233.9	28
^{13}C-PCB 81	non-Ortho PCB	17.72	301.9	231.9	28	303.9	233.9	28
PCB 81	non-Ortho PCB	17.73	289.9	219.9	28	291.9	221.9	28
^{13}C-PCB 77	non-Ortho PCB	18.04	301.9	231.9	28	303.9	233.9	28
PCB 77	non-Ortho PCB	18.05	289.9	219.9	28	291.9	221.9	28
^{13}C-2378-TCDF	PCDF	20.32	315.9	251.9	33	317.9	253.9	33
2378-TCDF	PCDF	20.34	303.9	240.9	33	305.9	242.9	33
^{13}C6-1234-TCDD	PCDD	20.44	325.9	262.9	28	327.9	264.9	28
^{13}C-2378-TCDD	PCDD	20.74	331.9	267.9	24	333.9	269.9	24
2378-TCDD	PCDD	20.75	319.9	256.9	24	321.9	258.9	24
^{13}C-PCB 126	non-Ortho PCB	20.93	335.9	265.9	28	337.9	267.9	28
PCB 126	non-Ortho PCB	20.95	323.9	253.9	28	325.9	255.9	28
^{13}C-12378-PeCDF	PCDF	23.29	351.9	287.9	35	349.9	285.9	35
12378-PeCDF	PCDF	23.29	339.9	276.9	35	337.9	274.9	35

			Quantifier			Qualifier		
Name	**Type**	**RT***	**Precursor ion**	**Product ion**	**CE†(V)**	**Precursor ion**	**Product ion**	**CE†(V)**
^{13}C-23478-PeCDF	PCDF	24.08	351.9	287.9	35	349.9	285.9	35
23478-PeCDF	PCDF	24.10	339.9	276.9	35	337.9	274.9	35
^{13}C-PCB 169	non-Ortho PCB	24.19	371.9	301.9	28	369.9	299.9	28
PCB 169	non-Ortho PCB	24.20	359.9	289.9	28	357.8	287.9	28
^{13}C-12378-PeCDD	PCDD	24.34	365.9	301.9	25	367.9	303.9	25
12378-PeCDD	PCDD	24.36	355.9	292.9	25	353.9	290.9	25
^{13}C-123478-HxCDF	PCDF	27.04	385.8	321.9	35	387.8	323.9	35
123478-HxCDF	PCDF	27.05	373.8	310.9	35	375.8	312.9	35
^{13}C-123678-HxCDF	PCDF	27.18	385.8	321.9	35	387.8	323.9	35
123678-HxCDF	PCDF	27.19	373.8	310.9	35	375.8	312.9	35
^{13}C-234678-HxCDF	PCDF	27.83	385.8	321.9	35	387.8	323.9	35
234678-HxCDF	PCDF	27.85	373.8	310.9	35	375.8	312.9	35
^{13}C-123478-HxCDD	PCDD	28.00	403.8	339.8	25	401.8	337.9	25
123478-HxCDD	PCDD	28.02	389.8	326.9	25	391.8	328.8	25
^{13}C-123678-HxCDD	PCDD	28.12	403.8	339.8	25	401.8	337.9	25
123678-HxCDD	PCDD	28.14	389.8	326.9	25	391.8	328.8	25
^{13}C-123789-HxCDD	PCDD	28.49	403.8	339.8	25	401.8	337.9	25
123789-HxCDD	PCDD	28.50	389.8	326.9	25	391.8	328.8	25
^{13}C-123789-HxCDF	PCDF	28.98	385.8	321.9	35	387.8	323.9	35
123789-HxCDF	PCDF	29.00	373.8	310.9	35	375.8	312.9	35
^{13}C-1234678-HpCDF	PCDF	31.13	419.8	355.8	36	421.8	357.8	36
1234678-HpCDF	PCDF	31.14	407.8	344.8	36	409.8	346.8	36
^{13}C-1234678-HpCDD	PCDD	32.97	437.8	373.8	25	435.8	371.8	25
1234678-HpCDD	PCDD	33.01	423.8	360.8	25	425.8	362.8	25
^{13}C-1234789-HpCDF	PCDF	33.97	419.8	355.8	36	421.8	357.8	36
1234789-HpCDF	PCDF	34.00	407.8	344.8	36	409.8	346.8	36
^{13}C-OCDD	PCDD	39.38	469.7	405.8	26	471.7	407.8	26
OCDD	PCDD	39.41	457.7	394.8	26	459.7	396.8	26
^{13}C-OCDF	PCDF	39.83	453.7	389.8	35	455.7	391.8	35
OCDF	PCDF	39.84	441.7	378.8	35	443.7	380.8	35

*RT=Retention time
†CE=Collision energy

Results and Discussion

Validation criteria

EU Regulation 709/2014 lists specific compliance requirements for GC/MS confirmatory methods for PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs [1]. Some of these criteria are dependent on the type of MS analyzer. For example, GC/triple quadrupole MS performs in tandem (MS/MS) mode, while GC/HRMS performs in selected ion monitoring (SIM) mode. Other requirements such as selectivity, upper- bound, and lower-bound differences are the same for both instrumental methodologies.

Each of these criteria was considered in this study, and a full method validation was performed for official control of dioxins in feed in accordance with the regulation, systematically investigating all parameters and performances on this instrumentation [1]. This validation could easily be transposed to other feed and foodstuffs, because it was performed using vegetable oil, which has maximum limits (MLs) that are amongst the lowest for these compounds (1.5 picograms WHO2005-PCDD/F-PCB-TEQ/g (parts per trillion)) [3], and the analytical criteria are the same. The World Health Organization 2005 toxic equivalent quantity per gram (WHO2005-TEQ) ML is the sum of the concentration of each individual congener corrected by a toxic equivalency factor (TEF) established by WHO in 2005. The measurement criteria for NDL-PCBs in food and feed are generally less stringent than those for PCDD/Fs and DL-PCBs since the MLs are in the ng/g (ppb) range and usually easier to attain [4]. However, for this validation study, the criteria for PCDD/Fs and DL-PCBs measurement were also applied to NDL-PCBs. For example, EU Regulation 709/2014 [5] stipulates only one precursor ion for quantitative and qualitative MRM transitions of NDL-PCBs, but two distinct precursor ions are required for PCDD/F and DL-PCB measurements. In this study, two specific precursor ions were also used for NDL-PCBs.

Instrumental limit of quantitation (iLOQ)

One of the major differences between the GC/triple quadrupole MS/MS and GC/HRMS methods is the proper establishment of the limit of quantitation (LOQ). Efficient ion filtering and the consequent significant reduction of noise are key advantages of GC/triple quadrupole MS/MS. A noise-free signal and flat baseline are characteristic of this instrumental approach. As a result, any signal-to-noise (S/N) calculation determined using such a baseline would produce S/N values that are unrealistic.

Therefore, a distinction was made for this validation study between a method limit of quantitation (mLOQ), which is the real-LOQ that takes possible matrix effects and blank levels into account, and the instrumental limit of quantitation (iLOQ), which is a performance- LOQ. This study used a statistical approach to assess the iLOQ of the GC/Triple Quadrupole MS/MS method, based on a report from an EU core working group composed of members from expert laboratories and EU national reference laboratories (NRLs) [6].

Eight replicate injections of the lowest acceptable calibration point were used to calculate the iLOQs, defined as 10 times the standard deviation (SD) associated with these replicates. To qualify as the lowest acceptable calibration point, the calculated relative standard deviations (RSDs) of the lowest level for all congeners had to be ≤15%. Although this 15% criterion was not included in Regulation 709/2014, it was chosen as a typical value for acceptable RSDs at such low analyte levels.

In addition, the regulation stipulates that the acceptable deviation to the relative response factor, which is the difference between the average response factors (RFs) obtained for the lowest calibration point versus the average RFs obtained for all points, is required to be ≤30%. The linearity of calibration was acceptable only when these two criteria were met. The iLOQ could then be determined as explained in the previous paragraph, using the resulting lowest calibration level.

Some exceptions were made in the calculation of the iLOQs shown in Table 3, when most criteria were acceptable. For example, 1,2,3,6,7,8-HxCDF had an RSD of 17.9% for triplicates of the lowest calibration point, which should have excluded it from calculation of iLOQ. However, the calibration coefficient (R^2) was very good (0.9990), and the difference between the average RF of the lowest point and the average RF of all points was only –1.21%, so a decision was made to use this lowest calibration point for the iLOQ calculation.

Table 3. iLOQs, Calibration Curve Data, and mLOQs

	iLOQ (pg/pL)	R^2	Lowest calibration point (pg/pL)	RSD of the lowest calibration point (%)	RF Difference (%)	Average blank level (ng/kg)	mLOQ (ng WHO2005 TEQ/kg)
PCDFs							
2,3,7,8-TCDF	0.010	0.9919	0.016	5.5	-11.82	33	0.010
1,2,3,7,8-PeCDF	0.022	0.9969	0.016	13.7	-9.38	92	0.017
2,3,4,7,8-PeCDF	0.021	0.9922	0.016	7.7	-3.25	8	0.025
1,2,3,4,7,8-HxCDF	0.016	0.9990	0.016	3.8	8.99	17	0.007
1,2,3,6,7,8-HxCDF	0.009	0.9990	0.016	17.9	-1.21	25	0.006
2,3,4,6,7,8-HxCDF	0.007	0.9993	0.016	9.3	7.17	33	0.008
1,2,3,7,8,9-HxCDF	0.020	0.9993	0.016	14.2	-7.19	50	0.018
1,2,3,4,6,7,8-HpCDF	0.053	0.9946	0.080	9.8	-8.94	92	0.005
1,2,3,4,7,8,9-HpCDF	0.020	0.9990	0.016	14.9	-2.23	25	0.000
OCDF	0.027	0.9933	0.016	12.4	18.92	83	0.000
						Sum mLOQ	0.096
PCDDs							
2,3,7,8-TCDD	0.018	0.9960	0.016	2.5	-1.94	0	0.005
1,2,3,7,8-PeCDD	0.029	0.9949	0.016	12.8	-3.72	0	0.007
1,2,3,4,7,8-HxCDD	0.022	0.9949	0.016	8.9	0.11	8	0.001
1,2,3,6,7,8-HxCDD	0.032	0.9996	0.040	4.6	8.23	25	0.014
1,2,3,7,8,9-HxCDD	0.062	0.9962	0.080	4.0	-6.60	17	0.004
1,2,3,4,6,7,8-HpCDD	0.053	0.9990	0.400	3.4	2.32	100	0.004
OCDD	0.465	0.9900	4.000	2.3	-11.83	100	0.001
						Sum mLOQ	0.036
						Sum PCDD/F mLOQ	0.132.
NO-PCBs							
PCB 81	0.030	0.9933	0.320	1.7	-10.65	75	0.001
PCB 77	0.037	0.9931	0.320	1.4	-10.31	100	0.005
PCB 126	0.077	0.9905	0.320	1.7	-9.96	92	0.137
PCB 169	0.071	0.9935	0.320	2.1	-7.19	0	0.001
						Sum mLOQ	0.144
MO-PCBs							
PCB-105	2.109	0.9958	1.000	9.8	-0.38	100	0.006
PCB-114	1.504	0.9938	1.000	8.8	-4.89	100	0.000
PCB-118	1.930	0.9994	1.000	8.7	15.32	100	0.018
PCB-123	1.537	0.9945	1.000	11.8	-4.86	100	0.000
PCB-156	1.897	0.9892	1.000	5.6	-5.38	100	0.000
PCB-157	1.287	0.9882	1.000	4.2	-7.09	100	0.000
PCB-167	2.067	0.9925	1.000	10.0	-6.32	100	0.001
PCB-189	1.626	0.9893	1.000	2.5	-7.48	100	0.000
						Sum mLOQ	0.025
						Sum PCDD/F-PCB mLOQ	0.300
NDL-PCBs							
PCB-28	3.928	0.9944	4.000	2.0	8.39	100	994.601*
PCB-52	6.530	0.9983	4.000	1.5	16.98	100	1909.234
PCB-101	2.733	0.9964	4.000	2.4	11.11	100	1303.266
PCB-138	1.587	0.9817	4.000	4.3	-5.59	100	161.674
PCB-153	1.469	0.9780	4.000	2.0	-6.03	100	171.672
PCB-180	0.904	0.9723	4.000	0.4	-1.64	100	34.941
						Sum mLOQ	4575.388

*Reported as ng/kg.

The iLOQs for the NDL-PCBs were much higher than those for the PCDDs, PCDFs, and non-ortho (NO-)PCBs, and some of them were higher than the iLOQs for the mono-ortho (MO-)PCBs as well (Table 3). This was due to the fact that the 1 pg/µL lowest calibration point for congeners was excluded from the iLOQ calculation for NDL-PCBs due to RF differences >30% and an RSD >15%. The 4 pg/µL calibration point was then used for all NDL-PCB iLOQ calculations, since all of its RSD values were far below 15%. Although the R^2 values were slightly below 0.9900 for three of the six NDL-PCBs (PCB-138,PCB-153, and PCB-180), the differences between RFs were very low, and the 4 pg/µL calibration point was used to calculate the iLOQs for these three NDL-PCBs as well. The calculated iLOQ values for all of the analytes were similar to those attained for GC/HRMS (data not shown).

Method limit of quantitation (mLOQ)

The mLOQ is a measure of the real analytical sensitivity of the method in a real environment. It is determined for the congeners by analyzing blank replicates, in this case 12 procedural blanks for each congener, from which an average value and an SD were calculated. The mLOQs are defined such that levels higher than the mLOQs are statistically proven to be due to the presence of congener in the sample, and not due to background noise. In this case, the mLOQs are defined as the average value of the blank plus six times the standard deviation.

Table 3 shows the average blank levels found in 12 individual procedure blanks for each of the 35 congeners, as well as mLOQs (ng WHO2005TEQ/kg). Most of the 35 congeners analyzed in our study, based on a 4-g sample size, gave measurable blank levels which were used to calculate the mLOQs (Table 3). For those congeners not present in blanks (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and PCB-169), the mLOQS were determined by adjusting the iLOQs to the sample amount.

The EU Regulation MLs are 0.75 ng/kg for the sum of the PCDDs and PCDFs (WHO2005-PCDD/F-TEQ/kg), 1.50 ng/kg for the sum of the PCDDs, PCDFs, NO-PCBS, and MO-PCBs (WHO2005-PCDD/F-PCB-TEQ/kg), and10 µg/kg for the sum of the 6 NDL-PCBs. The regulation requires that mLOQs must be <20% of MLs. Table 3 shows the sums of the mLOQs for each congener group for direct comparison with the MLs. The sum of 0.132 ng WHO2005-PCDD/F-TEQ/kg, is 18% of the ML, and the sum of 0.30 ng WHO2005-PCDD/F-PCB-TEQ/kg is 20% of the ML. This method is compliant with the regulation for these two congener groups. For the NDL-PCBs, the sum was 4.6 µg/kg, which was above 20% of the ML. This was due to a high level of contamination in the laboratory, as was also seen with the GC/HRMS method.

Selectivity and quantitative/qualitative transitions

The EU regulation does not call for specific criteria for the selectivity of GC/triple quadrupole MS/MS methods. However, triple quadrupole ion filtering produces a flat baseline, making these chromatograms look very different from those generated using GC/HRMS. To avoid any artificial enhancement of signals and keep as close as possible to raw data, unsmoothed chromatograms were used in this study. Baseline separation was observed for the 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorinated furans congeners (HxCDF), which are the most difficult to separate. No improvement in results was observed using smoothed chromatogram correction in terms of either accuracy or precision (RSD).

The intensity ratio of quantitation to qualification ions (Quant/Qual) was used to ensure absence of interference and correct peak integration. The lower response for an analyte was observed for the qualification transition (quantitation transition with a +2 Da offset), while the quantitation MRM transition gave the higher response. Using the same MS parameters such as collision energy, Quant/Qual ratios were established experimentally from the calibration curve (Table 4). The allowed tolerance was ±15% for PCDD/Fs and DL-PCBs, and more for NDL-PCBs [1]. To guarantee an accurate result, a closer look at raw data is required whenever a congener Quant/Qual ratio is out of range.

Table 4. Measured Average Quantitative/Qualitative Ion Ratio

	Mean	RSD%	Tolerance (%)
PCDFs			
2,3,7,8-TCDF	94.0	14	15
1,2,3,7,8-PeCDF	81.7	14	15
2,3,4,7,8-PeCDF	88.0	26	15
1,2,3,4,7,8-HxCDF	62.3	19	15
1,2,3,6,7,8-HxCDF	60.8	10	15
2,3,4,6,7,8-HxCDF	62.7	10	15
1,2,3,7,8,9-HxCDF	62.6	16	15
1,2,3,4,6,7,8-HpCDF	76.1	11	15
1,2,3,4,7,8,9-HpCDF	82.3	29	15
OCDF	93.0	20	15
PCDDs			
2,3,7,8-TCDD	96.4	10	15
1,2,3,7,8-PeCDD	81.6	21	15
1,2,3,4,7,8-HxCDD	64.7	15	15
1,2,3,6,7,8-HxCDD	64.4	17	15
1,2,3,7,8,9-HxCDD	73.3	19	15
1,2,3,4,6,7,8-HpCDD	79.7	18	15
OCDD	94.3	12	15
NO-PCBs			
PCB 81	64.3	14	15
PCB 77	62.4	1	15
PCB 126	95.1	9	15
PCB 169	73.3	8	15
MO-PCBs			
PCB-105	30.5	4	15
PCB-114	30.0	3	15
PCB-118	30.3	2	15
PCB-123	29.9	3	15
PCB-156	46.8	2	15
PCB-157	47.6	3	15
PCB-167	47.0	2	15
PCB-189	62.7	2	15
NDL-PCBs			
PCB-28	31.8	2	25
PCB-52	63.2	1	20
PCB-101	30.5	3	25
PCB-138	47.3	2	25
PCB-153	47.4	2	25
PCB-180	62.9	2	20

Background subtraction

Measured concentrations of an analyte can be corrected by subtracting an individual blank of the same kind of sample prepared in the same way. Such a blank is used for each series of samples (for example, one blank per 10 samples). Alternatively, an average blank value from a series of controlled blanks measured over time can be used for the correction. Two advantages are provided by the latter approach, which was used in this study. A chart of control levels kept over time enables detection of trends, can highlight contamination problems, and provides a proactive approach to contamination control. Secondly, this approach reduces the effect of a statistical outlier blank in a single sample series. Averaging blank levels that are controlled proactively within a confidence interval enables the rejection of such an outlier, and instead includes the statistical variation of the blank in the measurement uncertainty, which is monitored in the chart of control levels. This is a key point for determination of mLOQs.

Accuracy

The bias of the method for PCDD/Fs, NO-PCBs, and MO-PCBs was assessed using fortified samples in sunflower oil. No congener was found in the unfortified vegetable oil matrix blank. Six series of samples spiked at twice ML (2ML), ML, and half ML (ML/2) were injected over three days (two series per day), and all were within acceptable reproducibility limits (Table 5). The results were well within the requirements of the EU Regulation, which are method bias <20% and random error (%RSD) <15% [1].

Table 5. Bias of the Method for DL-PCBs and PCDD/Fs Using Six Series of Samples Spiked at Three Levels Around the ML

	Target*	Average*	SD	RSD%	Bias%
DL-PCBs					
2ML	1.3	1.26	0.02	1.6	–3.42
ML	0.65	0.59	0.02	3.4	–8.53
ML/2	0.33	0.31	0.03	9	–7.00
PCDD/Fs					
2ML	1.58	1.6	0.03	2.2	1.3
ML	0.79	0.78	0.04	5.7	–1.54
ML/2	0.4	0.41	0.03	7.1	2.36

* ng WHO2005TEQ/kg

Quality control and robustness

Two blank and two QC samples (pork fat) prepared in the lab were injected twice each week during September–October 2013, and again in April and May of 2014 (~6 months later). All of the QC sample values fell within the 95% confidence interval (Figure 1). That included QC samples that were run after the system was used for other purposes, including system venting and several column changes, over a 6-month period.

Conclusions

A GC/triple quadrupole MS/MS method has been developed and fully validated in accordance with criteria in EU Regulation 709/2014 that allows the use of GC/triple quadrupole MS/MS as a confirmatory method for official control of PCDDs, PCDFs, and DL-PCBs in animal feedstuffs. This method, developed using the Agilent 7890B GC system coupled to an Agilent 7000C Series Triple Quadrupole GC/MS system, meets the requirements of the regulation, and can achieve similar performance to GC/HRMS. Realistic measurement uncertainty in the typical range of the HRMS method was achieved, along with very similar analytical parameters, despite the difference in mass analyzer technology. The most stringent criteria were followed to demonstrate that this method provides accurate, consistent, and reliable results in the context of maximum level (ML) measurements.

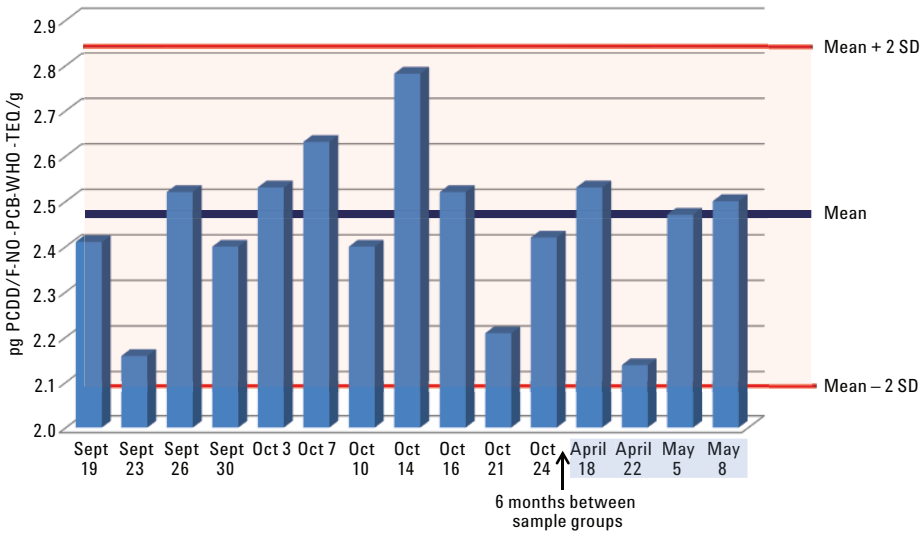

Figure 1. QC chart over September–October and April–May validation periods. All values fell within the 95% confidence interval (± 2SD.)

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Printed in the USA
January 19, 2016
5991-6590EN

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