Determination of Polychlorinated Biphenyl Congeners in Foodstuffs and Animal Feed using a Triple Quadrupole GC-MS/MS Instrument

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Introduction

Polychlorinated biphenyls (PCBs) are highly toxic, lipophilic Persistent Organic Pollutants (POPs) with properties that are detrimental to human health and have been linked to causing cancer, endocrine disruption and reproductive disorders. Until their ban in the late 20th Century, PCBs were widely manufactured for use in hundreds of industrial and commercial applications including electrical products and hydraulic equipment and as plasticizers in paints, plastics and, rubber products. PCB congeners that have been released into the environment can bio-accumulate in the tissues of animals and thereby enter the Human food chain.
Current legislation in the United States [1] and the European Union (EU) [2], [5] require the confirmation and quantitation of polychlorinated Dioxins (PCDD), polychlorinated Furans (PCDF) and Dioxin-like polychlorinated Biphenyl congeners (dl-PCBs) in foodstuffs and animal feed using isotope dilution capillary gas chromatography – high resolution mass spectrometry (GC-HRMS). The analysis of Dioxins and Furans in foodstuffs and animal feed by gas chromatography - triple quadrupole mass spectrometry is shown in a previously published Agilent application note [3].
Maximum levels for PCDD, PCDF and dl-PCB congeners in foodstuffs and animal feed are given in additional EU regulations [4], [6]. dl-PCB congeners have each been assigned a Toxic Equivalency Factor (TEF) which relates the toxicity of each individual dl-PCB congener to 2,3,7,8 Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) which itself is assigned a TEF of 1. The individual concentration of each dl-PCB found in foodstuffs and animal feed samples is multiplied by it's respective TEF and after summation the total concentration is expressed as the Toxic Equivalent (TEQ) in terms of pg TEQ/g fat, pg TEQ/g wet weight (fish) or ng TEQ/kg product based on 88% dry mass (feed).

This poster describes sensitive and reproducible GC-MS/MS methods for the screening of the 12 dl-PCBs (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) and the 6 non-dioxin-like PCB congeners (ndl-PCBs, # 28, 52, 101, 138, 153 and 180) in foodstuffs and animal feed using the Agilent 7000 Triple Quadrupole GC-MS/MS system that meets the requirements of current EU Legislation for a screening method.

Experimental

GC-MS/MS Methods

GC Conditions for Mono-*ortho* and ndl-PCB congeners

GC	Agilent 7890
Auto-sampler	Agilent 7693A
Column	50m x 0.22mm ID, 0.25um HT-8
Injection	2 µL cold splitless using CO2 cooled Multimode Inlet (MMI)
Injection port liner	4mm ID, un-packed
Inlet temperature program	100 °C (0.02 min), 500 °C/min to 300 °C
Purge Flow to Split Vent	50 mL/min at 1.0 min
Carrier Gas	Helium, constant flow 1.2 ml/min
Oven program	80°C (3.0 min hold), 20 °C/min to 160 °C (0 min), 4 deg °C/min to 300 °C (8 min) (Total run time = 50.0 minutes)
MS Transfer line temp	280 °C

GC Conditions for Non-*ortho* PCB congeners

Column	50m x 0.22mm ID, 0.25um HT-8
Injection	2 µL cold splitless using CO2 cooled Multimode Inlet (MMI)
Injection port liner	4mm ID with glass wool
Inlet temperature program	100 °C (0.02 min), 500 °C/min to 300 °C
Purge Flow to Split Vent	50 mL/min at 1.0 min
Carrier Gas	Helium, constant flow 1.2 ml/min
Oven program	120°C (2.0 min hold), 40 °C/min to 160 °C (0 min), 7 deg °C/min to 300 °C (10 min) (Total run time = 33.0 minutes)
MS Transfer line temp	280 °C

MS Conditions for all PCB congeners

Mass Spectrometer	Agilent 7000 triple quadrupole GC-MS/MS
Ion source temperature	280°C
Q1 / Q2 temperatures	150°C / 150°C
Q1 / Q2 Resolution	0.7u / 1.2u
Collison cell gas flows	Nitrogen 1.5 mL/min, Helium 2.25 mL/min
Electron energy	-78 eV
MS Tune file	Gain normalized Autotune
MS Gain setting	100

GC-MS/MS MRM Conditions:

Two different pre-cursor ions with two different product ions were monitored for all native dl- and ndl-PCBs and their ^{13}C-labelled internal standards. Full details of retention times, monitoring ions and optimized collison energies for the dl- and ndl-PCBs are given in Tables 1 and 2, respectively.

Experimental

TS	Segment Start Time (min.)	Analyte	RT (min.)	Quant Pre-cursor	Product	Dwell (ms)	CE (V)	Qual Pre-cursor	Product	Dwell (MS)	CE (V)
1	22.0	***^{13}C-PCB 28***	***24.34***	***268.0***	***198.1***	***25***	***26***	***270.0***	***198.1***	***25***	***26***
		PCB 28	***24.35***	***256.0***	***186.0***	***75***	***26***	***258.0***	***186.0***	***75***	***26***
		^{13}C-PCB 52	***25.66***	***302.0***	***232.0***	***25***	***28***	***304.0***	***234.0***	***25***	***28***
		PCB 52	***25.67***	***289.9***	***220.0***	***75***	***28***	***291.9***	***222.0***	***75***	***28***
2	29.0	***^{13}C-PCB 101***	***30.15***	***335.9***	***266***	***25***	***28***	***337.9***	***268.0***	***25***	***28***
		PCB 101	***30.16***	***323.9***	***253.9***	***75***	***28***	***325.9***	***255.9***	***75***	***28***
		^{13}C-PCB 123	33.55	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 123	33.56	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 118	33.76	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 118	33.77	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 141	34.00	371.9	301.9	25	28	369.9	299.9	25	28
		^{13}C-PCB 114	34.19	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 114	34.20	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 153	***34.50***	***371.9***	***301.9***	***25***	***28***	***369.9***	***299.9***	***25***	***28***
		PCB 153	***34.51***	***359.8***	***289.9***	***75***	***28***	***357.8***	***287.9***	***75***	***28***
		^{13}C-PCB 105	35.15	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 105	35.16	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 138	***35.88***	***371.9***	***301.9***	***25***	***28***	***369.9***	***299.9***	***25***	***28***
		PCB 138	***35.89***	***359.8***	***289.9***	***75***	***28***	***357.8***	***287.9***	***75***	***28***
		^{13}C-PCB 167	37.64	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 167	37.65	359.8	289.9	75	28	357.8	287.9	75	28
3	38.5	^{13}C-PCB 156	38.78	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 156	38.79	359.8	289.9	75	28	357.8	287.9	75	28
		^{13}C-PCB157	39.06	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 157	39.07	359.8	289.9	75	28	357.8	287.9	75	28
		^{13}C-PCB 180	***39.17***	***407.8***	***337.9***	***25***	***30***	***405.8***	***335.9***	***25***	***30***
		PCB 180	***39.18***	***393.8***	***323.9***	***75***	***30***	***395.8***	***325.9***	***75***	***30***
		^{13}C-PCB 189	42.43	407.8	337.9	25	30	405.8	335.9	25	30
		PCB 189	42.44	393.8	323.9	75	30	395.8	325.9	75	30

Table 1: Retention times and MS/MS transitions for mono-*ortho* and ndl-PCB congeners (ndl-PCB congeners shown in *red bold italics*) and ^{13}C-Internal standards

TS	Segment Start Time (min.)	Analyte	RT (min.)	Quant Pre-cursor	Product	Dwell (ms)	CE (V)	Qual Pre-cursor	Product	Dwell (ms)	CE (V)
1	19	^{13}C-PCB 81	20.74	301.9	232.0	25	28	303.9	234.0	25	28
		PCB 81	20.75	289.9	220.0	125	28	291.9	222.0	125	28
		^{13}C-PCB 77	21.12	301.9	232.0	25	28	303.9	234.0	25	28
		PCB 77	21.13	289.9	220.0	125	28	291.9	222.0	125	28
2	22	^{13}C-PCB 126	23.55	335.9	265.9	25	28	337.9	267.9	25	28
		PCB 126	23.56	323.9	253.9	125	28	325.9	255.9	125	28
3	25	^{13}C-PCB 169	26.26	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 169	26.27	359.9	289.9	125	28	357.8	287.9	125	28

Table 2: Retention times and MS/MS transitions for non-*ortho* PCB congeners and ^{13}C-Internal standards

Sample Preparation

The most frequently used methods for the determination of PCDD, PCDF, dl-PCBs and ndl-PCBs in foodstuffs and animal feed combine fat extraction (e.g. Soxhlet or extraction with organic solvents) with clean up steps using different column chromatographies such as silica gel coated with sulphuric acid, Florisil, alumina and, active carbon. The final extracts are collected as 3 fractions containing the Mono-*ortho* PCB congeners and Indicator PCB congeners (1a, Figure 1), non-*ortho* PCB congeners (1b, Figure 1) and PCDD/F (2, Figure 1), by eluting with various solvents. After addition of a syringe spike of ^{13}C- labelled PCB internal standards, the extracts were evaporated under a gentle stream of nitrogen and subsequently reconstituted with toluene and analyzed with GC-MS/MS. The PCDD/F fraction was reconstituted with 20 µL toluene, the non-*ortho* PCB fraction with 40 µL toluene and the Mono-*ortho* / Indicator PCB fraction with 250 µL toluene. A flow diagram summarizing the sample preparation steps is shown in Figure 1.

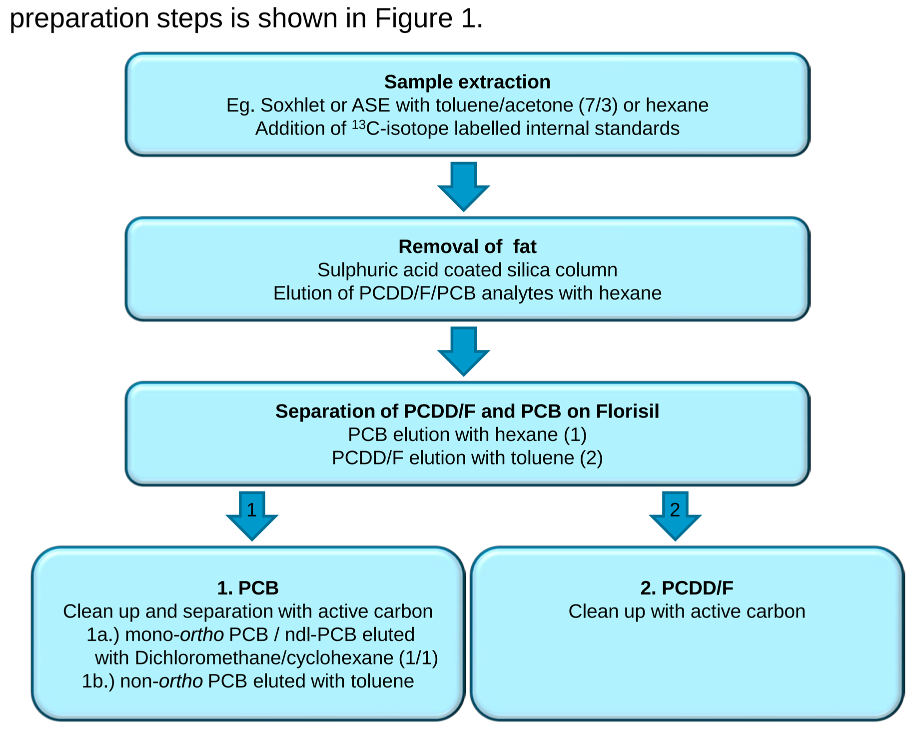

Figure 1 : Flow diagram of the sample extraction and clean-up procedures

Results and Discussion

The multiple reaction monitoring (MRM) chromatograms for the native Mono-*ortho* PCB congeners,and ndl-PCB congeners, with an analysis time of 50 minutes, are shown in Figure 2. The MRM chromatograms for the native Non-*ortho* PCB congeners, with an analysis time of 33 minutes, are shown in Figure 3.

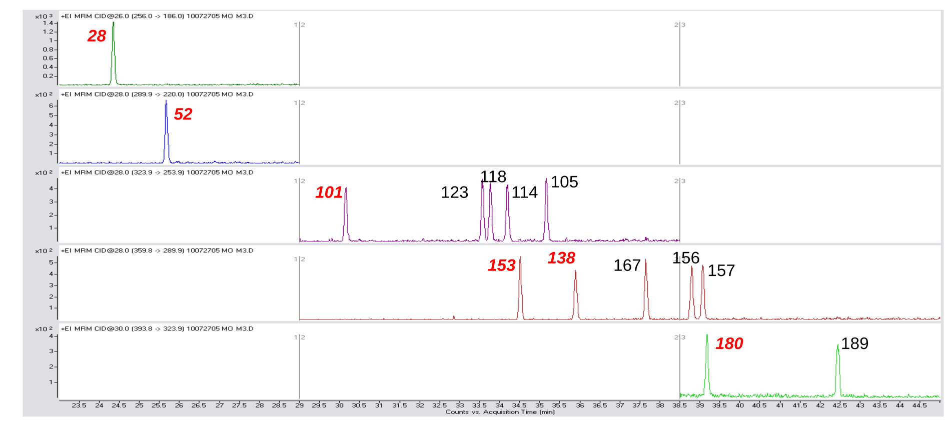

Figure 2. MRM chromatograms of native Mono-*ortho* PCB congeners and ndl-PCB congeners (ndl-PCB congeners labelled in *red bold italics*)

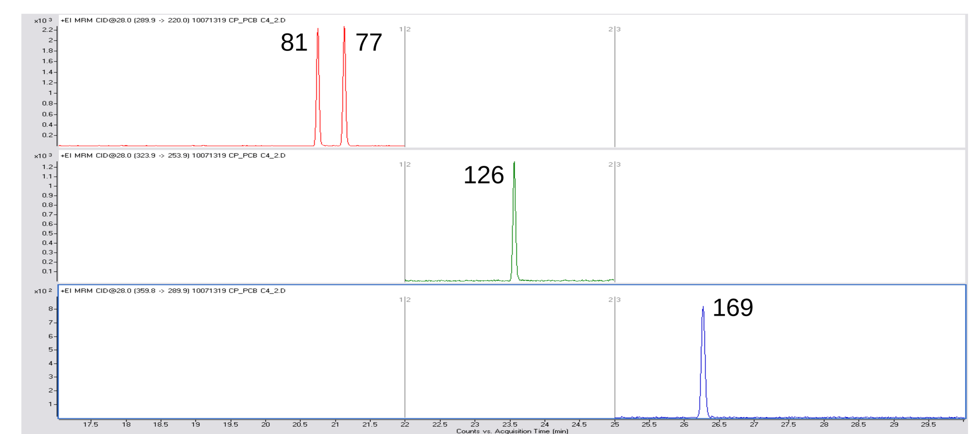

Figure 3. MRM chromatograms of native Non-*ortho* PCB congeners

Linearity of Response

All PCB congeners were measured using ^{13}C-labelled internal standard (ISTD) calibration. The ^{13}C-labelled ISTDs were added at the start of the extraction process. 7-point ISTD calibration curves were created over the range of 0.05 pg/µL – 50.0 pg/µL for the Mono-*ortho* PCB congeners and 0.10 pg/µL – 10.0 pg/µL for the Non-*ortho* PCB congeners. An example calibration curve for PCB 126 is shown in Figure 4.The linear calibration curve fits for all the PCB congeners are shown in Table 3, all analytes gave linear curve fit coefficients (R^2) greater than 0.998.

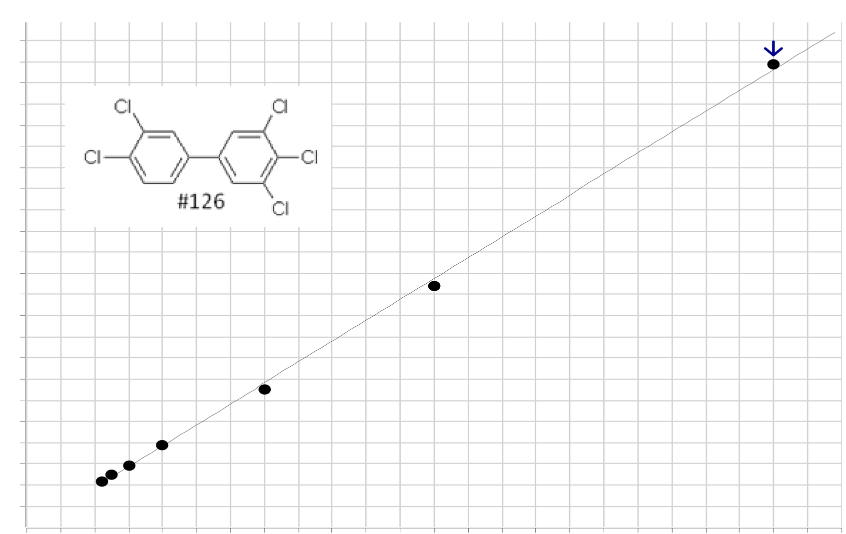

Figure 4. Linear calibration curve for PCB 126 over the concentration range 0.10 pg/µL – 10 pg/µL

Mono-*ortho* PCBs	R^2	Non-*ortho* PCBs	R^2
PCB 28	0.9999	PCB 81	0.9992
PCB 52	0.9993	PCB 77	0.9991
PCB 101	0.9991	PCB 126	0.9991
PCB 123	0.9997	PCB 169	0.9999
PCB 118	0.9994		
PCB 114	0.9998		
PCB 153	0.9997		
PCB 105	0.9999		
PCB 138	0.9993		
PCB 167	0.9988		
PCB 156	0.9985		
PCB 157	0.9987		
PCB 180	0.9995		
PCB 189	0.9990		

Table 3. Linear correlation coefficients for 7 point ISTD calibration curves over the range 0.05 pg/µL – 50 pg/µL for Mono-*ortho* PCB congeners and 0.1 pg/µL – 10 pg/µL for Non-*ortho* PCB congeners, injection volume = 2µL

Results and Discussion

Sample Analysis

In order to assess the quantitative performance of the GC-MS/MS system, 80 samples of four different foodstuffs and feed, Animal Feed (n=45), Cows' milk (n=11), Meat (n=19) and Liver (n=5) 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 Triple Quadrupole GC /MS/MS system and reanalyzed.

Figure 5 shows the comparative sample results (Total TEQ-dl-PCB, Upperbound values) for the two sets of measurements expressed as the percentage difference between the results obtained by the GC-HRMS and GC-MS/MS analyses. The x-axis is the quantitative result for each sample and the y-axis shows the percentage difference between the quantitative results measured on the GC-MS/MS system and on the GC-HRMS system.

Quantitaive results were calculated using WHO_{98} Toxic Equivalency Factors (TEF_{WHO98}) and reported as TEQ_{WHO98} values (sum of dl-PCB pg/g fat).

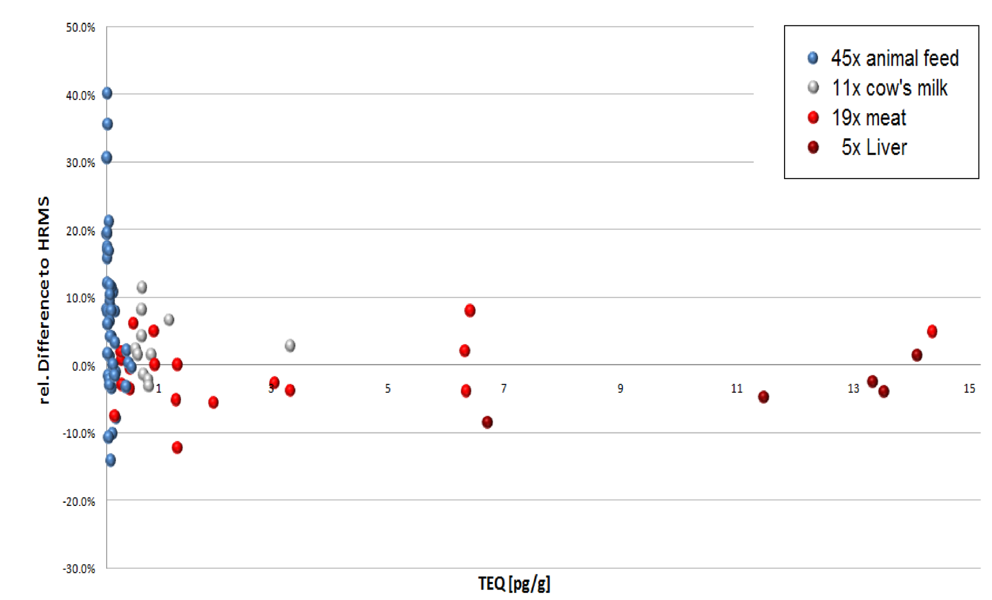

Figure 5. Comparative results for the sum of the 12 dl-PCB Congeners (TEQ_{WHO98} Upperbound values) for 80 Foodstuffs and Animal Feed samples analyzed by GC-HRMS and GC-MS/MS

The agreement between the results obtained for the total of the 12 dl-PCB congeners on the GC-HRMS and the GC-MS/MS system for foodstuffs and animal feed samples at levels above 1 pg TEQ/g were within the range of +/- 10 %.

The comparative results for the 68 foodstuffs and animal feed samples that gave total dl-PCB results less than 1.2 TEQ pg/g are shown in Figure 6.

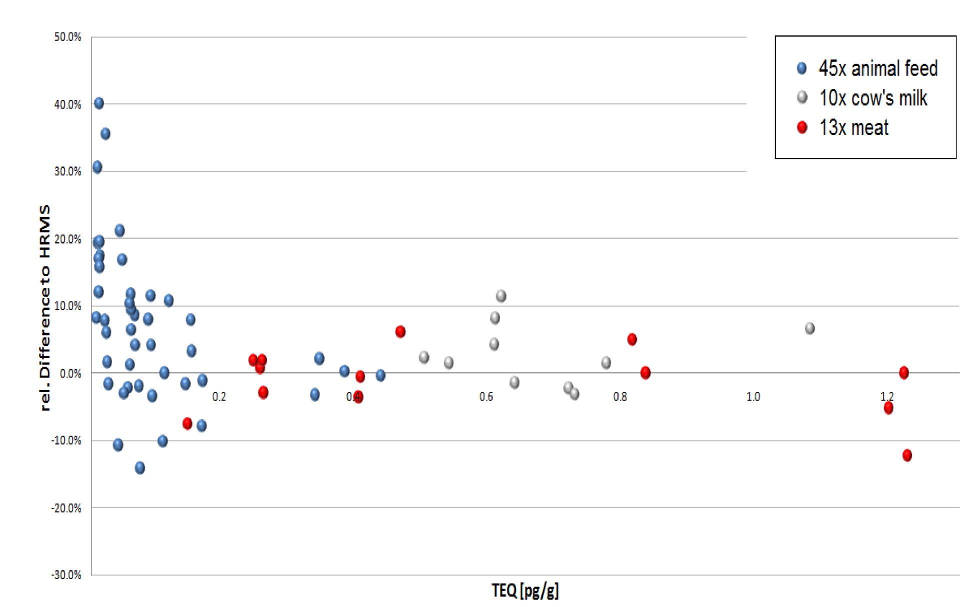

Figure 6: Comparative results for the sum of the 12 dl-PCB Congeners (TEQ_{WHO98} Upperbound values) for 68 Foodstuffs and Animal Feed samples analyzed by GC-HRMS and GC-MS/MS which gave values less than ~1.2 pg TEQ/g product

The agreement between the results obtained for the sum of the 12 dl-PCB congeners on the GC-HRMS and the GC-MS/MS system for foodstuffs and animal feed samples at levels between 0.1 and 1 pg TEQ/g was within the range of +/- 15 %. Only those animal feed samples with total dl-PCB congener concentrations below 0.1 pg TEQ/g gave some results with percentage differences greater than 15%.

Results and Discussion

The comparative sample results (Total ndl-PCB congeners, Upperbound values) for the two sets of measurements expressed as the percentage difference between the results obtained by the GC-HRMS and GC-MS/MS analyses for 67 Foodstuffs and Animal Feed samples which gave values less than10 ng/g product are shown in Figure 7.

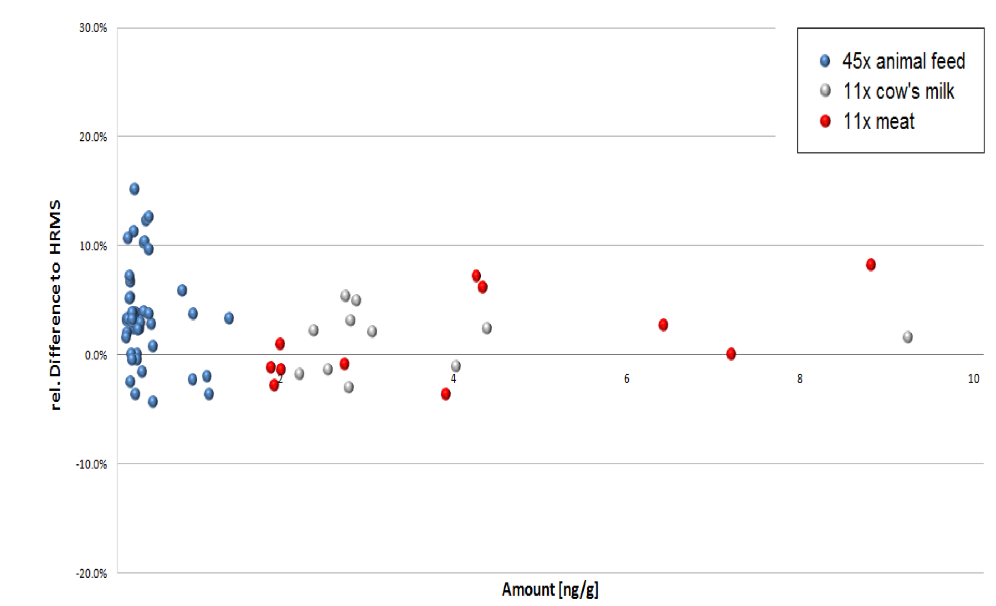

Figure 7: Comparative Results for the sum of 6 ndl-PCB congeners (Upperbound values) for 67 Foodstuffs and Animal Feed samples analyzed by GC-HRMS and GC-MS/MS which gave values less than 10 ng/g product

The agreement between the sum of the results obtained for the 6 ndl-PCB congeners with the GC-HRMS and the GC-MS/MS for foodstuffs and animal feed samples at levels between 0.5 and 10 ng/g was within the range of +/- 10 %. Some Animal Feed samples with total ndl-PCB congener concentrations below 0.5 ng/g gave results with percentage differences greater than +10%.

Conclusions

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

Additionally, the Agilent 7000 Triple Quadrupole GC-MS/MS system has been shown to be able to determine total ndl-PCB congeners in foodstuffs and animal feed samples at concentration levels of 1ng/g product and below, also in good agreement with results obtained by GC-HRMS.

References

(1) EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment and Tissue by HRGC/HRMS.

(2) 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.

(3) C Sandy, "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", Agilent Technologies publication 5990-6594EN (2010).

(4) COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 Setting maximum levels for certain contaminants in foodstuffs.

(5) COMMISSION REGULATION (EC) No 152/2009 Annex V letter B of 27 January 2009, Laying down the methods of sampling and analysis for the official control of feed.

(6) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002, on undesirable substances in animal feed.