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WP 071

Trace Analysis of Dioxins and Dioxin-Like PCBs Utilizing GC/MS/MS with a New High Efficiency Source

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are highly toxic Persistent Organic Pollutants (POPs) with properties that are detrimental to human health and have been linked to cancer, endocrine disruption, and reproductive disorders. Many of these compounds are not intentionally produced, but are created as by-products of industrial processes, pesticide manufacturing, combustion processes, and other sources. Even though these toxic compounds are not deliberately produced; their chemical properties impart their stability in the environment. These compounds are lipophilic chemicals that accumulate in the fatty tissues of animals that form part of the food chain.

Since these toxic compounds enter and remain in the human food chain, they are monitored and regulated by Food Researchers and Environmental Agencies (such as the European Commission (EC), US EPA, and WHO).

As of June 2014 the European Union (EU) has instituted new regulations governing the levels of PCDDs, PCDFs, dioxin-like (DL; including non-ortho (NO) and mono-ortho (MO) substituted) PCBs and non-dioxin-like (NDL) PCBs in food and feed. In a new amendment to EU legislations *No. 589/2014* and *No. 709/2014*, the use of GC/MS/MS systems has been accepted as a *confirmatory* technique for checking compliance with maximum levels (ML). Previously, the use of a High Res Mass Spectrometry (HRMS) was needed to confirm and quantify dioxins, due to the ability to identify, confirm, and quantitate the trace levels of dioxins. With the use of a new high-efficiency Electron Ionization (EI) source incorporated in the 7010 Triple Quadrupole GC/MS system, the GC/MS/MS can now confidently detect and quantitate dioxins and dioxin-like PCBs at ultra-trace levels.

Experimental

Sample Preparation

The most frequently used methods for the determination of PCDD/PCDF and DL-PCB in foodstuffs and animal feed combine fat extraction (i.e. Soxhlet) with cleanup steps using different column chromatographies (i.e. silica gel coated with sulphuric acid, florisil, alumina, and active carbon)

Manual dioxin sample preparation is tedious and comprehensive; multicolumn automated systems have been made to automate dioxin sample extraction to reduce analysis times and to attempt to reduce costs

The final extract is collected as two separate fractions:
1) PCDDs/PCDFs and the NO-PCB congeners
2) MO-PCB congeners and the NDL-PCB congeners

Along with the analysis of the native compounds: 13C-ISTDs are required for each individual standard for accurate identification and quantitation; Surrogates are also added prior to extraction to correct for analyte recovery

Simple straight forward configuration

Analysis utilizes:

Agilent 7010 MS/MS – Increased sensitivity
Consistent GC method – Increased productivity
MMI inlet – Flexibility with injection techniques/volumes
GC column – Validated for dioxin analysis
RT locked MRM transitions – Optimized & validated

GC Conditions			
Column	DB 5MSUI 60 m x 0.25 mmID x 0.25 µm		
Injection port liner	2mm id dimpled splitless liner, UI		
Injection mode	Cold-splitless (compressed air/CO_2 cooled MMI)		
Injection volume	1 µL		
Inlet temperature program		60 °C	0.31 min
	600 °C/min	330 °C	5 min
Carrier gas	He, constant flow 1.00 mL/min		
Oven program		60 °C	1 min
	30 °C/min	270 °C	1 min
	2 °C/min	310 °C	0 min
	5 °C/min	350 °C	0.5 min
MS transfer line temperature	350 °C		

High-Efficiency EI Source (Agilent 7010 Triple Quadrupole GC/MS)

MS sensitivity depends on the number of ions measured

This new ultra-efficient EI source maximizes the number of ions that are created and transferred out of the source body and into the quadrupole analyzer

Advantages:

- Increased response and better precision at all levels
- Lower detection limits
- More precise ion ratios and better qualitative information

Unit mass resolution allows for sufficient resolution to separate two peaks one mass unit apart

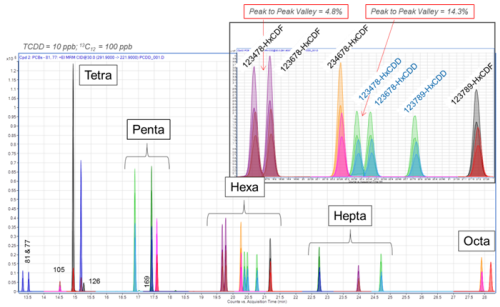
As well as the ability to minimize possible interferences on the analytes of interest

MS/MS parameters	
Electron Energy	70 eV
Tune	eihs.tune.xml
EM gain	10
MS1 resolution	Unit
MS2 resolution	Unit
Collision Cell	1.5 mL/min N_2 4 mL/min He
Quant/Qual transitions	Fraction Specific
Dwell times	Fraction Specific
Collision energies	Optimized
Source temperature	350 °C
Quad temperatures	150 °C

Meeting the EU Criteria

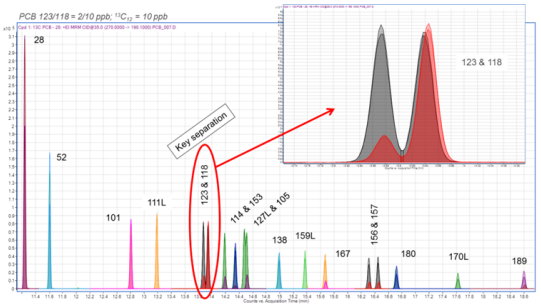
Dioxins/Furans – Chromatogram

Excellent separation of the difficult hexa-dioxin/furan isomers



PCBs – Chromatogram

Key separation between the difficult MO-PCBs 123 & 118



7010 MS/MS Instrument Detection Limit (IDL_{RSD}) in fg

$$IDL_{RSD} = \frac{t_{\alpha,n-1} \times RSD \times c}{100}$$

$t_{\alpha,n-1}$ = t value (coefficient) at the level of α with the sample size of n-1

c = concentration of the std sample injected

CMPD	RRF	10 reps (CS1)	
		% RSD	IDL_{RSD} (fg)
2378-TCDF	1.180	4.92	6.8
2378-TCDD	1.258	4.28	5.9
12378-PeCDF	1.206	2.39	16.5
23478-PeCDF	0.961	2.98	20.6
12378-PeCDD	1.080	3.91	27.0
123478-HxCDF	1.278	3.33	23.0
123678-HxCDF	1.194	2.58	17.8
234678-HxCDF	1.171	2.71	18.7
123478-HxCDD	1.184	4.83	33.4
123678-HxCDD	1.183	4.40	30.4
123789-HxCDD	1.178	4.92	34.0
123789-HxCDF	1.906	2.24	15.5
1234678-HpCDF	1.183	2.54	17.6
1234678-HpCDD	1.171	3.37	23.3
1234789-HpCDF	0.875	5.44	37.6
OCDD	1.391	3.69	51.0
OCDF	1.963	3.04	42.0

CMPD	RRF	10 reps (CS1)	
		% RSD	IDL_{RSD} (fg)
PCB – 28	1.077	1.58	22.3
PCB – 52	1.465	1.91	26.9
PCB – 101	1.276	1.57	22.1
PCB – 77	1.040	1.41	4.0
PCB – 81	1.024	1.71	4.8
PCB – 118	2.854	4.18	11.8
PCB – 123	0.620	1.43	20.2
PCB – 105	3.316	9.87	27.8
PCB – 114	0.883	1.97	27.8
PCB – 153	0.671	18.48	52.1
PCB – 138	1.402	1.17	16.5
PCB – 126	1.061	5.43	15.3
PCB – 167	1.168	2.11	6.0
PCB – 156	1.053	4.24	12.0
PCB – 157	1.025	3.49	9.8
PCB – 180	0.930	1.24	17.5
PCB – 169	1.228	2.12	6.0
PCB – 189	1.095	3.13	8.8

Instrumental limit of quantitation (iLOQ) A 'Performance LOQ'

(10 replicate injections @ lowest calibration point)

iLOQ = 10 x StdDev

MDL = (Student's t value at 99% confidence level) x StdDev

Dioxin/Furan	StdDev	RSD %	MDL (fg)	iLOQ (fg)
2378-TCDF	0.003	4.92	8	28
2378-TCDD	0.003	4.28	7	25
12378-PeCDF	0.007	2.39	19	66
23478-PeCDF	0.008	2.98	24	84
12378-PeCDD	0.011	3.91	31	108
123478-HxCDF	0.009	3.33	26	93
123678-HxCDF	0.007	2.58	21	73
234678-HxCDF	0.008	2.71	21	76
123478-HxCDD	0.013	4.83	37	132
123678-HxCDD	0.013	4.40	36	126
123789-HxCDD	0.014	4.92	41	144
123789-HxCDF	0.006	2.24	18	63
1234678-HpCDF	0.007	2.54	20	72
1234678-HpCDD	0.010	3.37	28	100
1234789-HpCDF	0.016	5.44	44	157
OCDD	0.022	3.69	61	217
OCDF	0.017	3.04	48	170

PCB	StdDev	RSD %	MDL (fg)	iLOQ (fg)
PCB – 28	1.077	1.58	23	80
PCB – 52	1.465	1.91	26	94
PCB – 101	1.276	1.57	22	77
PCB – 81	1.040	1.41	4	15
PCB – 77	1.024	1.71	5	17
PCB – 123	2.854	4.18	17	60
PCB – 118	0.620	1.43	20	71
PCB – 114	3.316	9.87	32	115
PCB – 153	0.883	1.97	27	96
PCB – 105	0.671	18.48	296	1050
PCB – 138	1.402	1.17	16	57
PCB – 126	1.061	5.43	15	55
PCB – 167	1.168	2.11	6	22
PCB – 156	1.053	4.24	12	43
PCB – 157	1.025	3.49	10	37
PCB – 180	0.930	1.24	17	61
PCB – 169	1.228	2.12	6	22
PCB – 189	1.095	3.13	9	32

Ion Ratios at lowest calibration point (iLOQ)

Dioxin/Furan	AVG	StdDev	%RSD (±15%)
2378-TCDF	1.10	0.07	6
2378-TCDD	0.95	0.04	4
12378-PeCDF	0.80	0.02	3
23478-PeCDF	0.79	0.02	3
12378-PeCDD	0.79	0.05	6
123478-HxCDF	0.64	0.03	4
123678-HxCDF	0.63	0.03	5
234678-HxCDF	0.64	0.04	5
123478-HxCDD	0.57	0.05	9
123678-HxCDD	0.60	0.06	10
123789-HxCDD	0.59	0.03	6
123789-HxCDF	0.63	0.02	3
1234678-HpCDF	0.79	0.04	5
1234678-HpCDD	1.21	0.06	5
1234789-HpCDF	1.24	0.05	4
OCDD	0.99	0.04	4
OCDF	1.07	0.03	3

PCB	AVG	StdDev	%RSD (±15%)
PCB – 28	0.63	0.01	1
PCB – 52	0.64	0.01	1
PCB – 101	1.11	0.02	2
PCB – 81	0.64	0.01	2
PCB – 77	0.65	0.01	2
PCB – 123	0.86	0.08	9
PCB – 118	0.96	0.03	3
PCB – 114	0.86	0.09	10
PCB – 153	0.79	0.02	3
PCB – 105	0.95	0.01	1
PCB – 138	0.78	0.01	1
PCB – 126	1.00	0.09	9
PCB – 167	0.78	0.02	2
PCB – 156	0.81	0.04	5
PCB – 157	0.81	0.06	7
PCB – 180	0.64	0.01	2
PCB – 169	0.80	0.03	4
PCB – 189	0.64	0.02	4

Recovery – Measuring Precision and Accuracy

(TCDD spike at 0.79pg-TEQ/g fat)

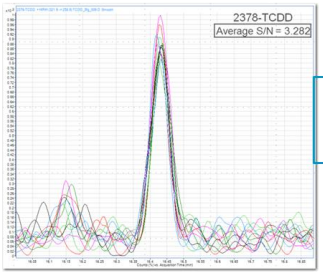
Compound	REP_1	REP_2	REP_3	REP_4	REP_5	REP_6	Avg.	StdDev	RSD%
PCB 81	2.230	2.228	1.902	1.899	1.958	2.024	**2.040**	**0.15**	**7.5**
PCB 77	5.568	5.602	5.590	5.611	5.630	5.528	**5.588**	**0.04**	**0.6**
PCB 126	1.677	1.684	1.687	1.751	1.684	1.490	**1.662**	**0.09**	**5.3**
PCB 169	1.415	1.387	1.341	1.396	1.397	1.351	**1.381**	**0.03**	**2.1**
2378-TCDF	0.082	0.088	0.083	0.087	0.095	0.084	**0.087**	**0.00**	**5.5**
2378-TCDD	0.086	0.076	0.077	0.076	0.071	0.084	**0.078**	**0.01**	**7.2**
12378-PeCDF	0.083	0.087	0.090	0.085	0.087	0.106	**0.090**	**0.01**	**9.3**
23478-PeCDF	0.077	0.080	0.078	0.085	0.083	0.089	**0.082**	**0.00**	**5.6**
12378-PeCDD	0.081	0.067	0.091	0.082	0.078	0.078	**0.080**	**0.01**	**9.8**
123478-HxCDF	0.086	0.081	0.081	0.089	0.086	0.104	**0.088**	**0.01**	**9.7**
123678-HxCDF	0.080	0.075	0.085	0.078	0.090	0.089	**0.083**	**0.01**	**7.4**
234678-HxCDF	0.076	0.061	0.076	0.075	0.071	0.069	**0.071**	**0.01**	**8.2**
123478-HxCDD	0.069	0.079	0.070	0.073	0.069	0.067	**0.071**	**0.00**	**6.1**
123678-HxCDD	0.093	0.101	0.089	0.078	0.090	0.100	**0.092**	**0.01**	**9.2**
123789-HxCDD	0.067	0.069	0.072	0.062	0.057	0.067	**0.066**	**0.01**	**8.2**
123789-HxCDF	0.076	0.085	0.084	0.094	0.084	0.074	**0.083**	**0.01**	**8.7**
1234678-HpCDF	0.386	0.361	0.391	0.388	0.415	0.451	**0.399**	**0.03**	**7.7**
1234678-HpCDD	0.121	0.119	0.122	0.121	0.149	0.122	**0.126**	**0.01**	**9.1**
1234789-HpCDF	0.025	0.025	0.025	0.027	0.029	0.028	**0.027**	**0.00**	**6.6**
OCDD	0.481	0.461	0.484	0.475	0.486	0.486	**0.479**	**0.01**	**2.0**
OCDF	0.166	0.160	0.157	0.174	0.174	0.182	**0.169**	**0.01**	**5.6**
								0.02	**6.7**

Discussion

Pushing the 7010 MS/MS IDL

10 x 1 µL cold-splitless injections of 5fg/µL TCDD were analyzed
(S/N was determined by RMS with a multiplier of 5)

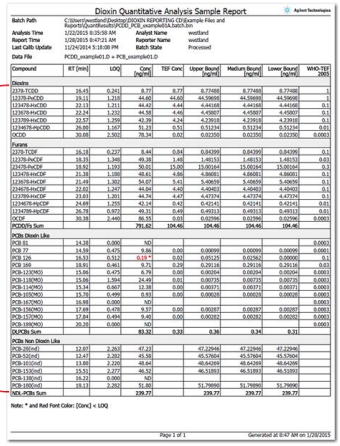
CMPD	RRF	% RSD	IDL_{RSD} (fg)
2378-TCDF	1.239	8.99	1.3
2378-TCDD	1.379	11.33	1.6
12378-PeCDF	1.247	3.96	2.8
23478-PeCDF	1.072	4.15	2.9
12378-PeCDD	1.120	4.29	3.0
123478-HxCDF	1.300	7.81	5.5
123678-HxCDF	1.185	6.56	4.6
234678-HxCDF	1.247	4.53	3.2
123478-HxCDD	1.213	8.02	5.7
123678-HxCDD	1.175	9.76	6.9
123789-HxCDD	1.277	6.88	4.8
123789-HxCDF	1.908	6.70	4.7
1234678-HpCDF	1.274	5.15	3.6
1234678-HpCDD	1.278	7.79	5.5
1234789-HpCDF	0.977	7.93	5.6
OCDD	0.761	7.56	10.7
OCDF	1.064	5.16	7.3



Customized Reporting

Customized reporting software makes the complicated calculations automated and provides a detailed report!

Combines both sample fraction runs into ONE consolidated report!

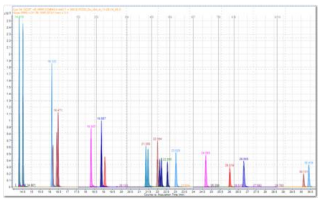


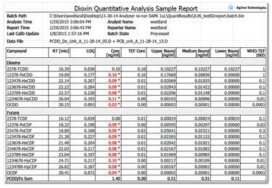
Results

Dioxins in Food and Animal Feed Samples

Unknown samples were analyzed for dioxins and dioxin-like PCBs

Unknown A





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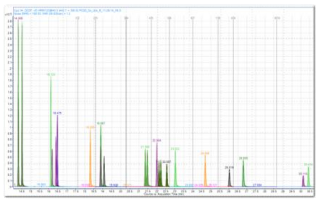

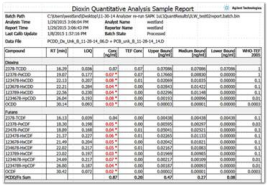

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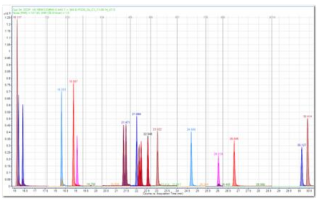

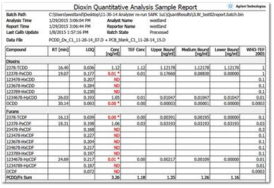

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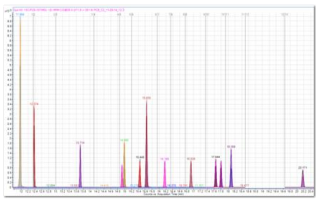

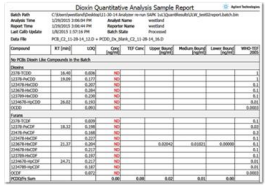

Conclusions

A complete workflow is demonstrated based upon the use of GC/MS/MS as a confirmatory technique for checking compliance with MLs of PCDDs, PCDFs, and dioxin-like PCBs in food and feed. The application was developed, optimized, and validated in conjunction with the new EU legislations *No. 589/2014* and *No. 709/2014*.

The use of the Agilent 7010 GC/MS/MS Triple Quadrupole is not only a cost effective alternative to HRMS, but it also provides:

- A multi mode inlet – offers a wider range of injection techniques and injection volumes to maximize detection limits,
- Same GC parameters (for both sample fractions) – increases productivity
- Retention time locking – time segment boundaries for acquisition and quantitation methods are maintained
  - MRM transitions – developed and optimized for the 7010 GC/MS/MS
- The new high efficiency source – increased sensitivity!
- Customized reporting – combining results from dioxin/furan and dioxin-like PCB fractions and automatically delivers TEF results and other complex calculations required by the EU regulations

All of these advantages provide for a complete workflow for the analysis of dioxin/furan and dioxin-like PCB in food and animal feed per the new amendments to the EU legislation.