Development and Validation of an Alternate Procedure for PCDD/PCDF. Adaptation of HRMS Method 1613B Protocols and Criteria to MS/MS

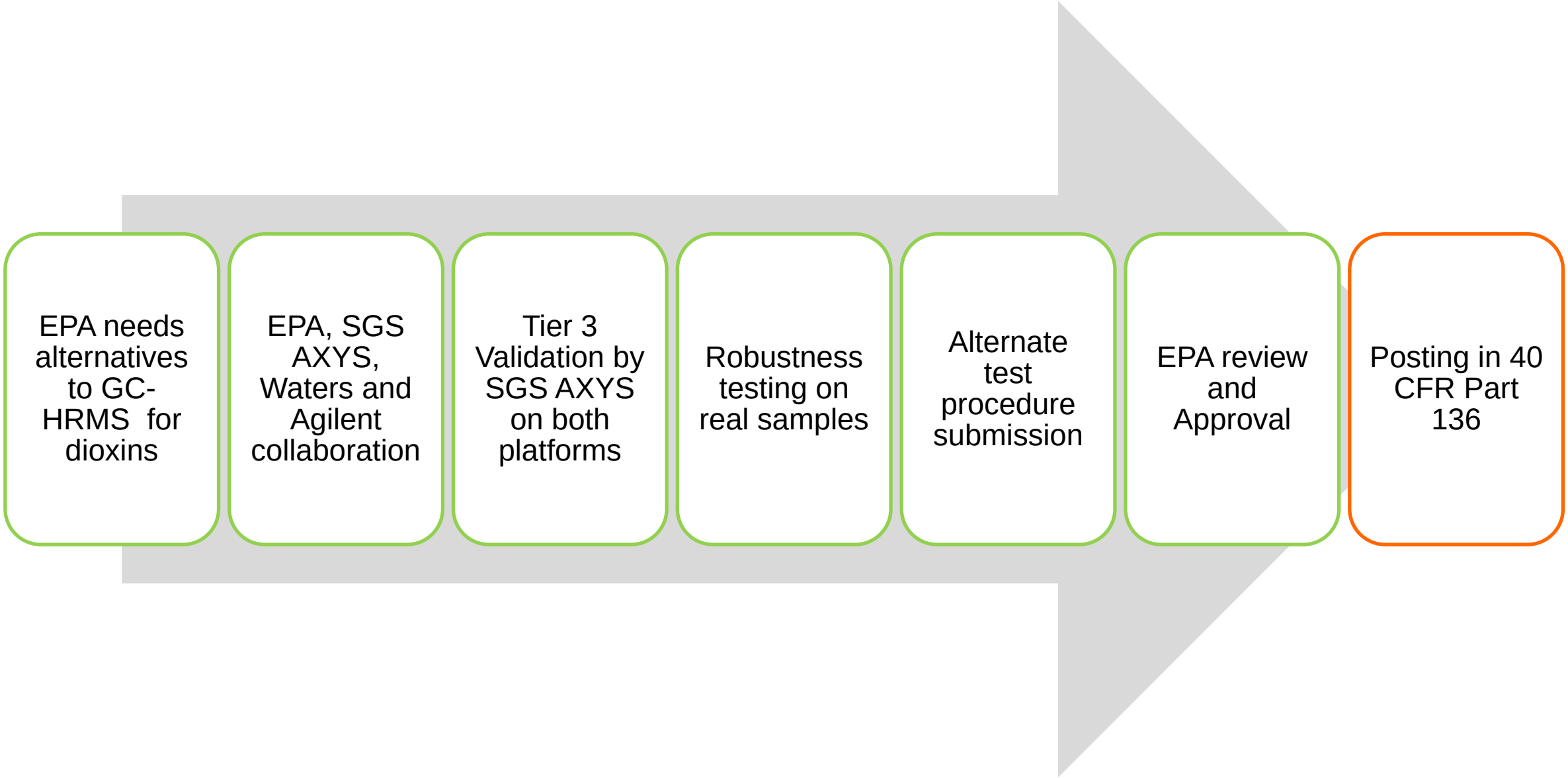
Coreen Hamilton

SGS AXYS Analytical Services Ltd.

ACKNOWLEDGEMENTS

- Lem Walker, EPA Office of Water, ATP Coordinator
- Joe Romano (Waters) and Tarun Anumol (Agilent)
- SGS AXYS Senior Chemists; Xinhui Xie, Angie Schlak, Kristen Bowes and Robert Tones, each with decades of experience with HRMS analyses

SGS AXYS METHOD 16130



QUESTIONS

- Why is a new method needed?
- Does MS/MS have the required sensitivity?
- Does MS/MS have the required selectivity?
- Do other environmental contaminants interfere?
- Can the detector system handle the matrix background in real samples?

SGS AXYS

DEVELOPMENT OF A METHOD FOR PCDD/F ANALYSIS BY GC-MS/MS

- Focus only on detector system (MS/MS)
- Verify operating parameters: Instrument set up and control, MRM transitions, product ions ratios
- Confirm QC criteria that are not HRMS-specific, e.g. sensitivity, linearity
- Adapt HR-specific QC Protocols to MS/MS e.g. detector specificity and stability (mass resolution and lock mass) monitoring, qualitative identification criteria
- Investigate potential interferences – PCBs, chlorodiphenyl ethers
- Monitor Performance on Real Samples

MRM TRANSITIONS AND RATIOS

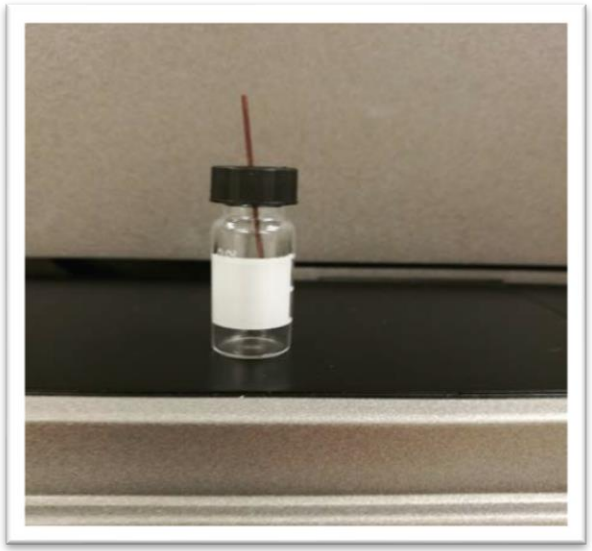
Species Monitored.	Precursor m/z Primary/Secondary	Transition Product[3] Ion Theoretical Ratio[4]	QC Limit [1]	
			Lower	Upper
Cl_4CDD [2]	(M+2)/M	0.96	0.82	1.10
Cl_4CDF	(M+2)/M	0.96	0.82	1.10
Cl_5CDD	M/(M+2)	0.78	0.66	0.90
Cl_5CDF	M/(M+2)	0.78	0.66	0.90
Cl_6CDD	(M+4)/(M+2)	0.64	0.54	0.74
Cl_6CDF	(M+4)/(M+2)	0.64	0.54	0.74
Cl_7CDD	(M+4)/(M+2)	0.80	0.68	0.92
Cl_7CDF	(M+4)/(M+2)	0.80	0.68	0.92
Cl_8CDD	(M+4)/(M+2)	0.96	0.82	1.10
Cl_8CDF	(M+4)/(M+2)	0.96	0.82	1.10

- QC limits represent ±15% windows around the theoretical ion abundance ratios.
- Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (clean-up standard).
- Product ions are due to loss of [$CO^{35}Cl$].
- Transition Product ion ratios are calculated as secondary ion/primary ion.

USE OF A REFERENCE COMPOUND TO MONITOR DETECTOR RESPONSE STABILITY

- To detect suppression of detector response by sample matrix – replacement for HRMS lock mass monitoring requirement
- Bleed a reference compound into the detector system throughout run and monitor its response. Expect it to be constant.
- Select PFTBA (the tuning compound). Large number of masses available.
- Select 414.0 > 264.0 transition due to good response and appropriate mass range. Monitor 264.0 response continuously.

USE OF A REFERENCE COMPOUND TO MONITOR DETECTOR RESPONSE STABILITY



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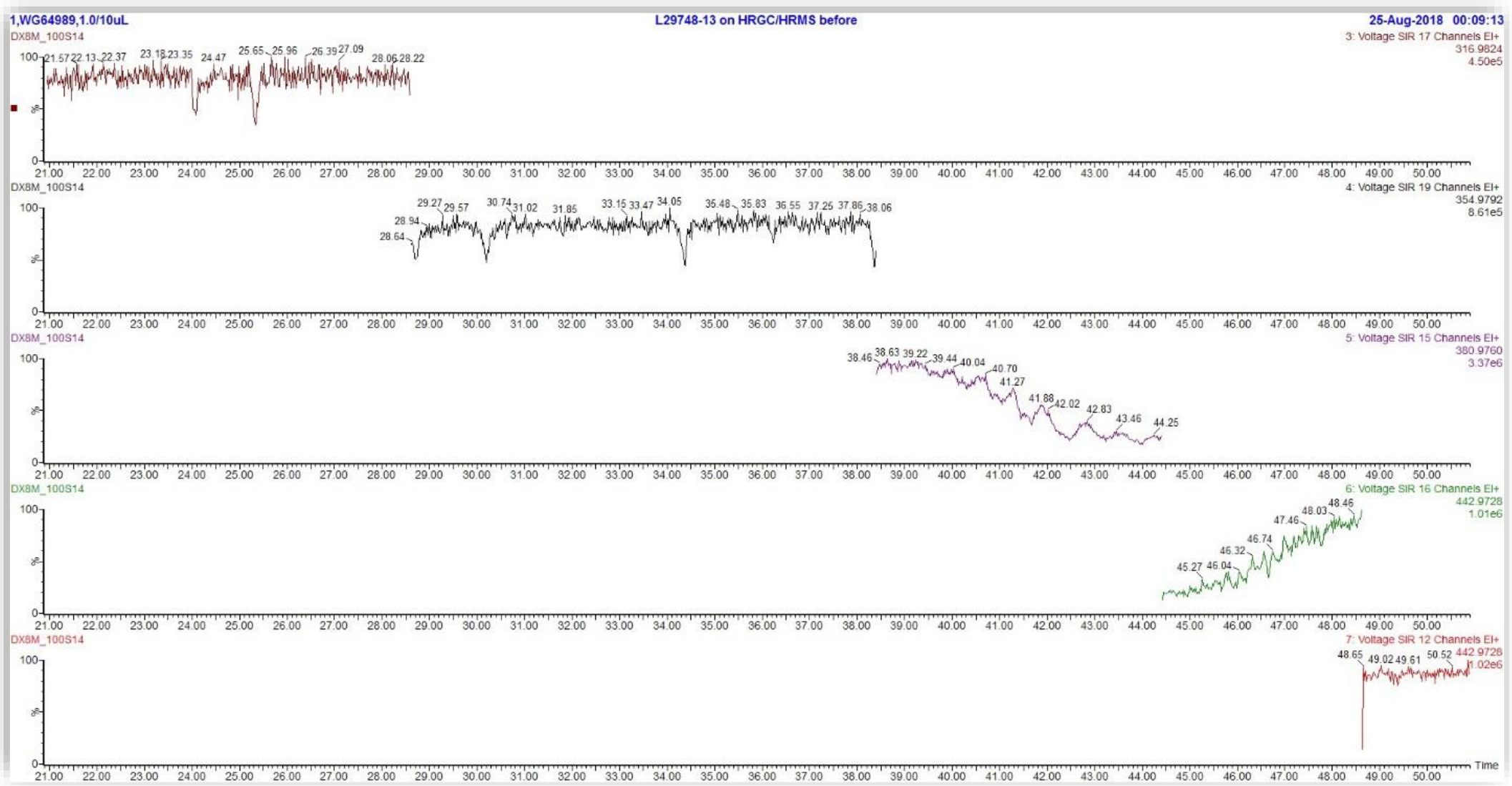
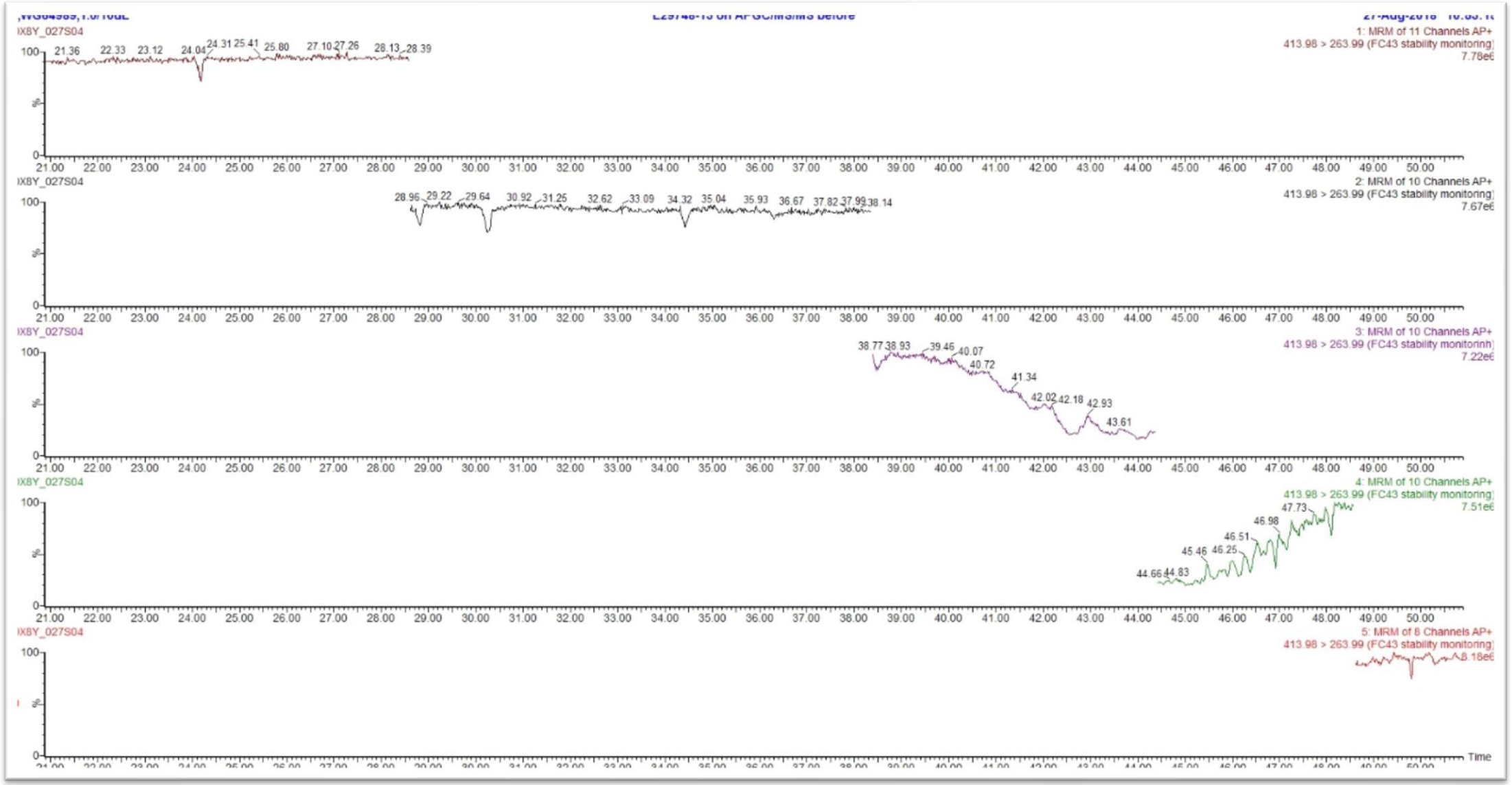
HRMS ANALYSIS OF “DIRTY” SEDIMENT EXTRACT-LOCK MASS IONS IN 5 FUNCTIONS



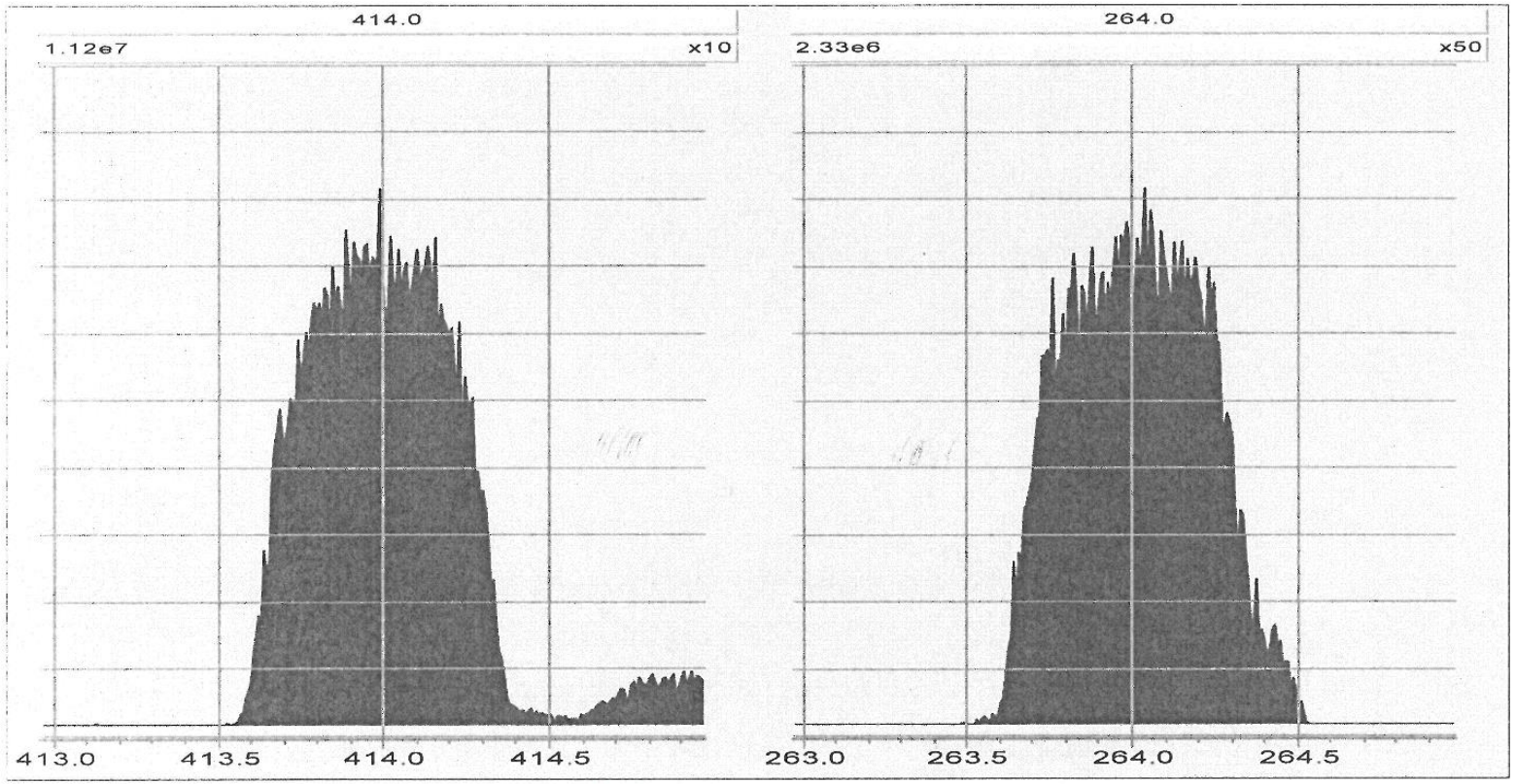
MS/MS REFERENCE COMPOUND RESPONSE IN 5 FUNCTIONS



HRMS LOCK MASS FUNCTIONS AFTER EXTRA SAMPLE CLEANUP

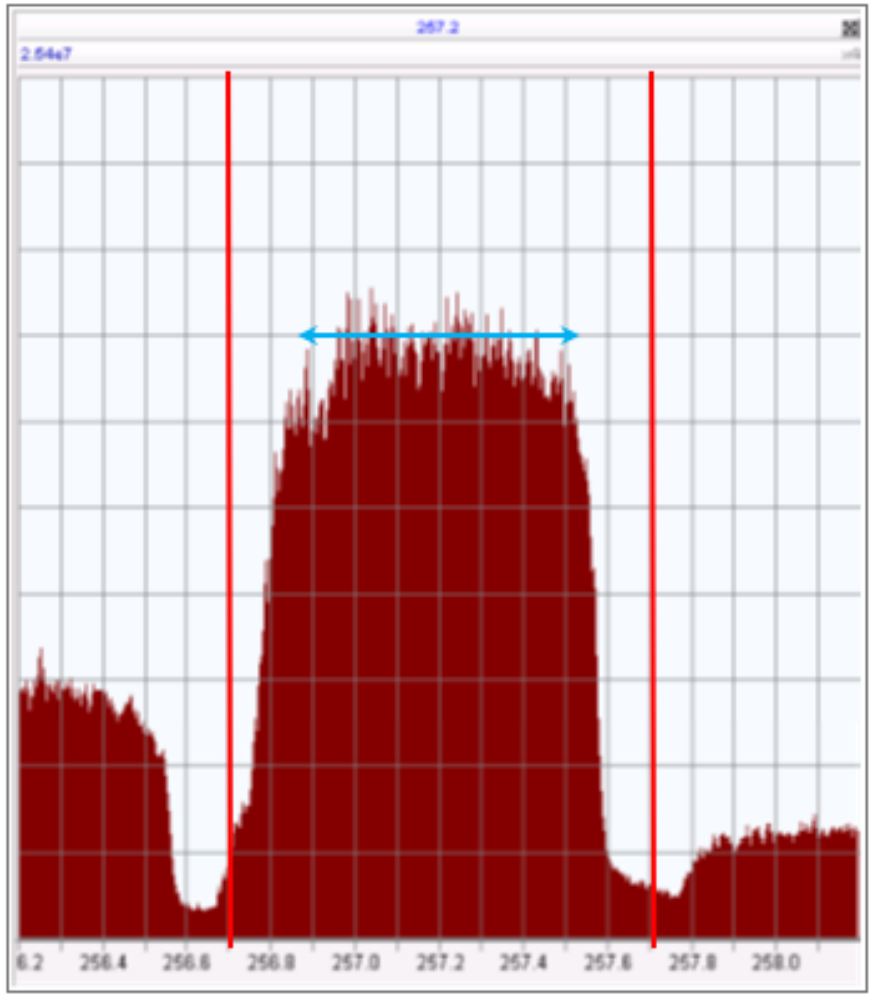
MS/MS REFERENCE COMPOUND RESPONSE AFTER EXTRA SAMPLE CLEANUP

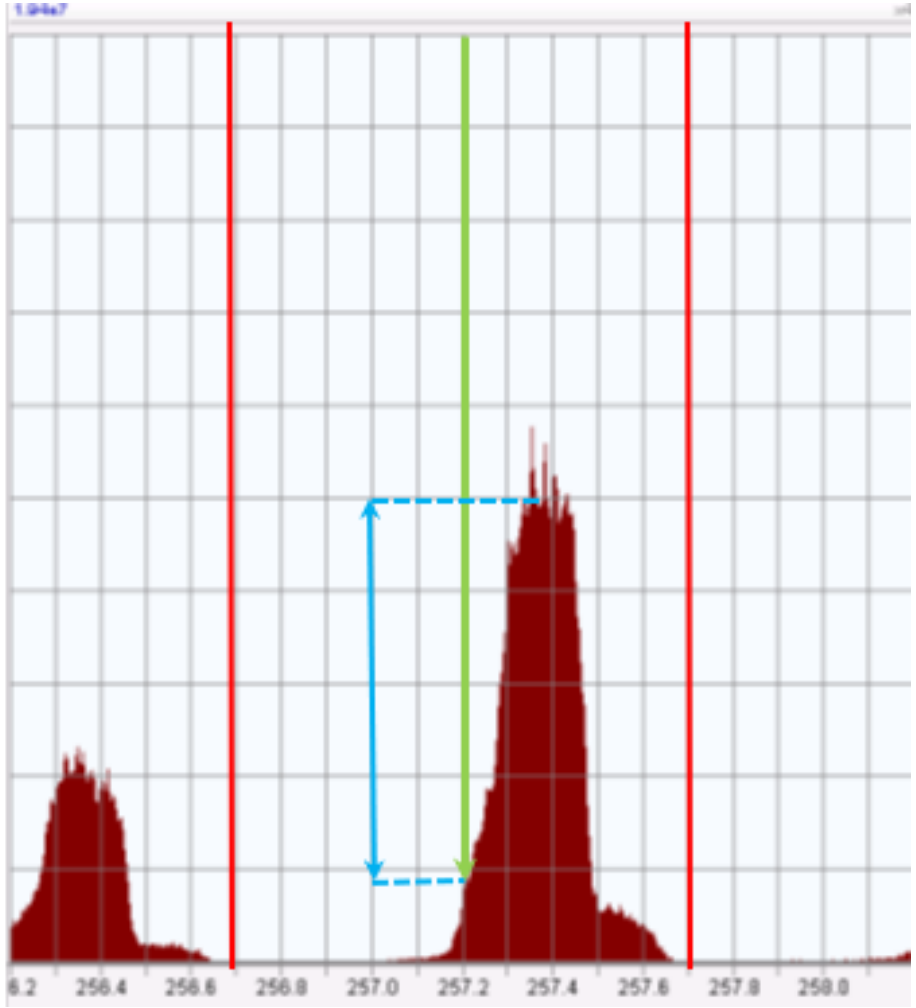
MASS RESOLUTION AND MASS ACCURACY



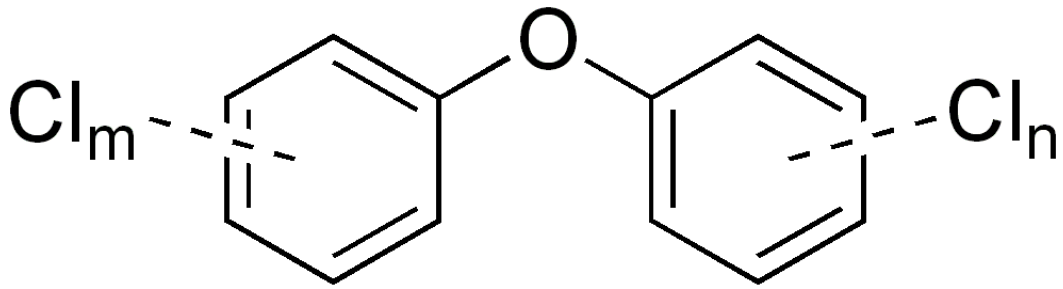
- Demonstrate unit mass resolution and mass accuracy on an on-going basis.
- How: PFTBA transition 414.0 > 264.0. Scan MS1 and MS2
- Every 12 hours
- Criteria for resolution: resolution of 414.0 from 415.0 and peak width at base is 1 ± 0.2 amu

MASS RESOLUTION:
NOT TOO MUCH, NOT TOO LITTLE. JUST RIGHT



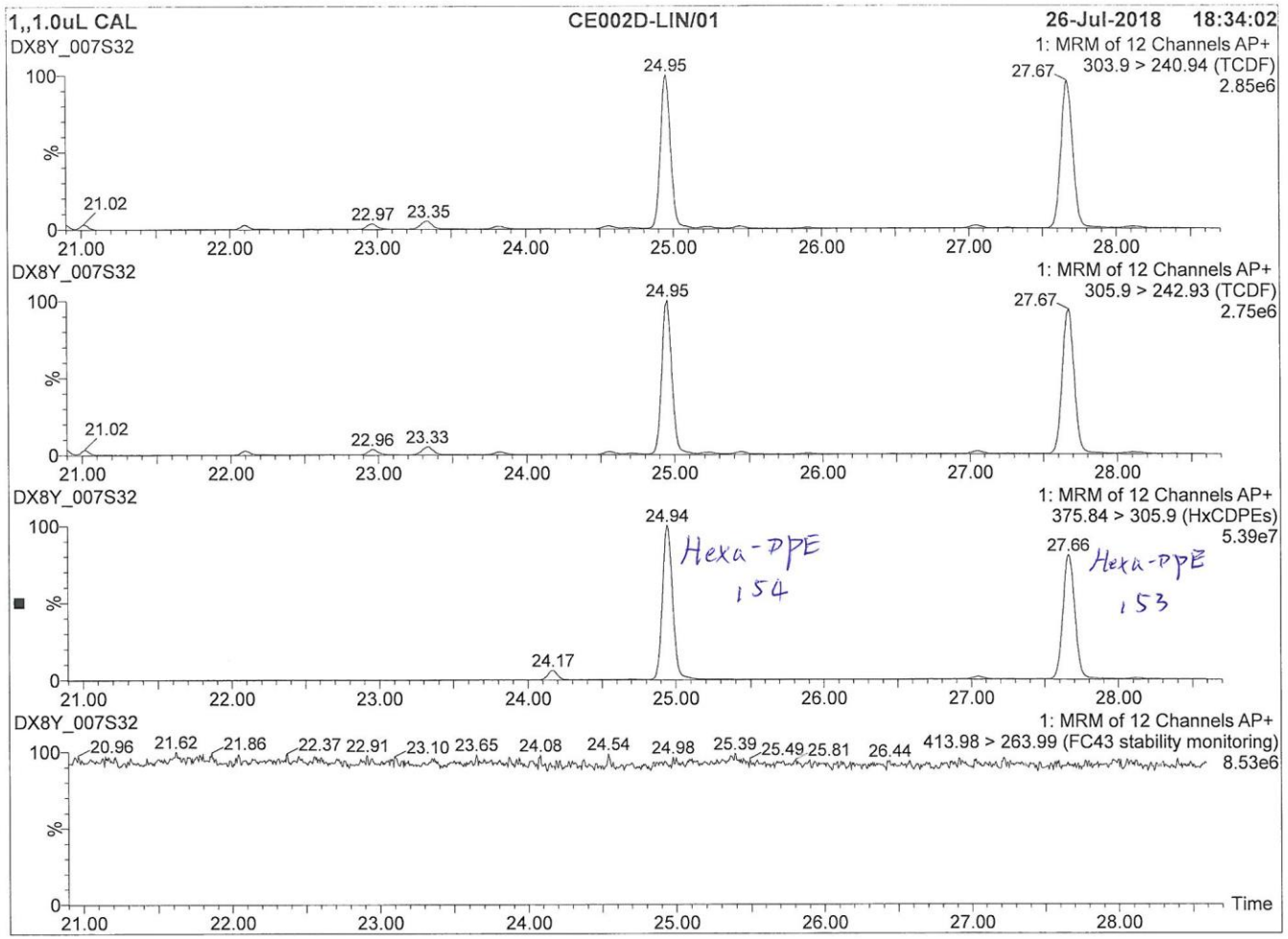


CAN CHLORODIPHENYL ETHERS INTERFERE?

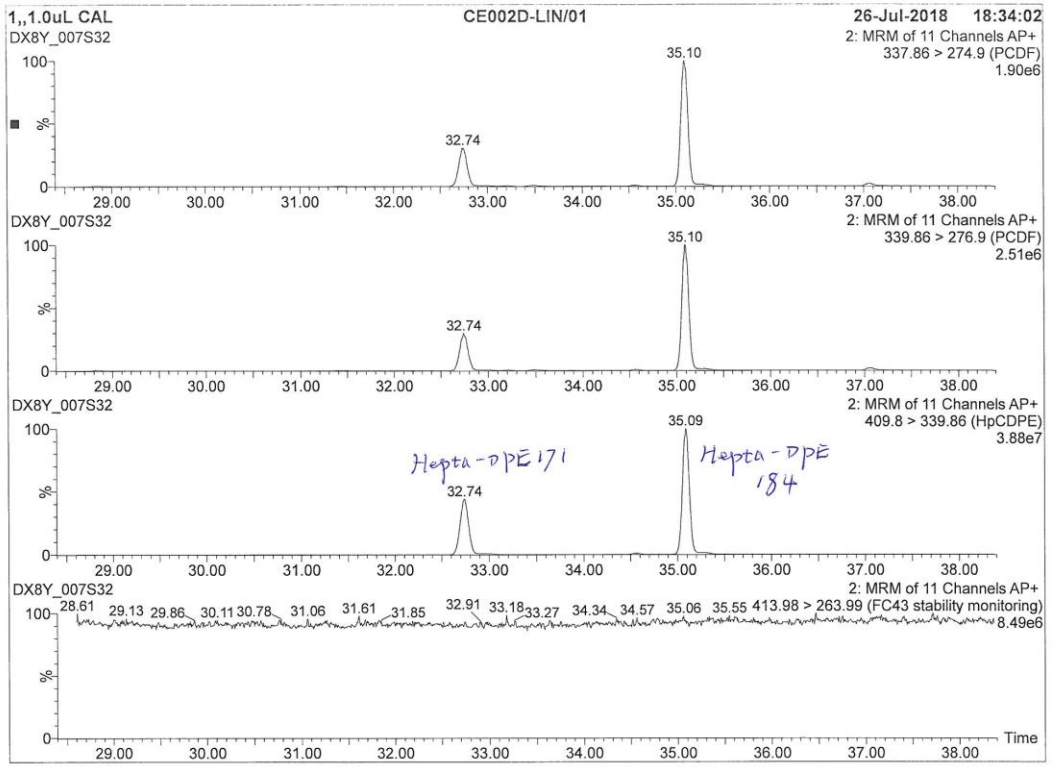


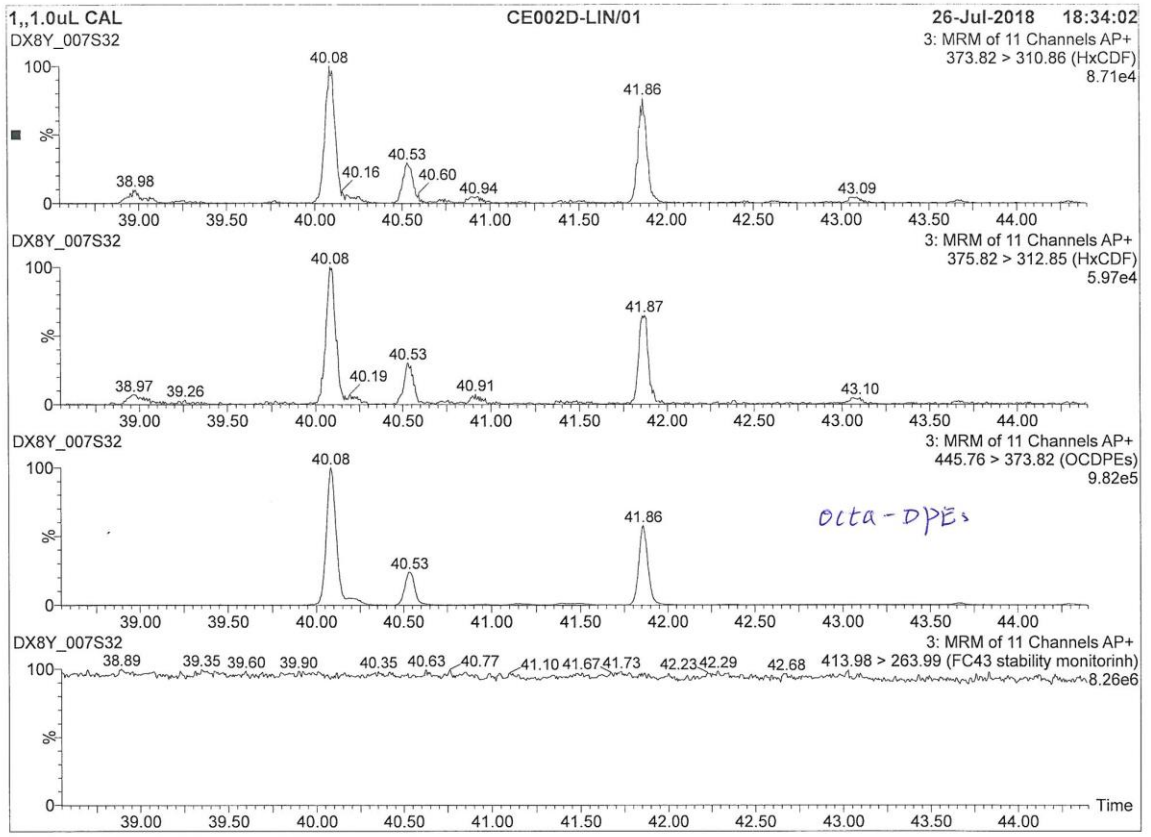
- Run Chlorodiphenyl ether standards and acquire PCDF data
- Response for selected diphenyl ethers observed in PCDF channels
- Magnitude of response depends on ionization conditions
- Monitor chlorodiphenyl ether MRMs in each function (as in HRMS)

HEXACHLORODIPHENYL ETHERS

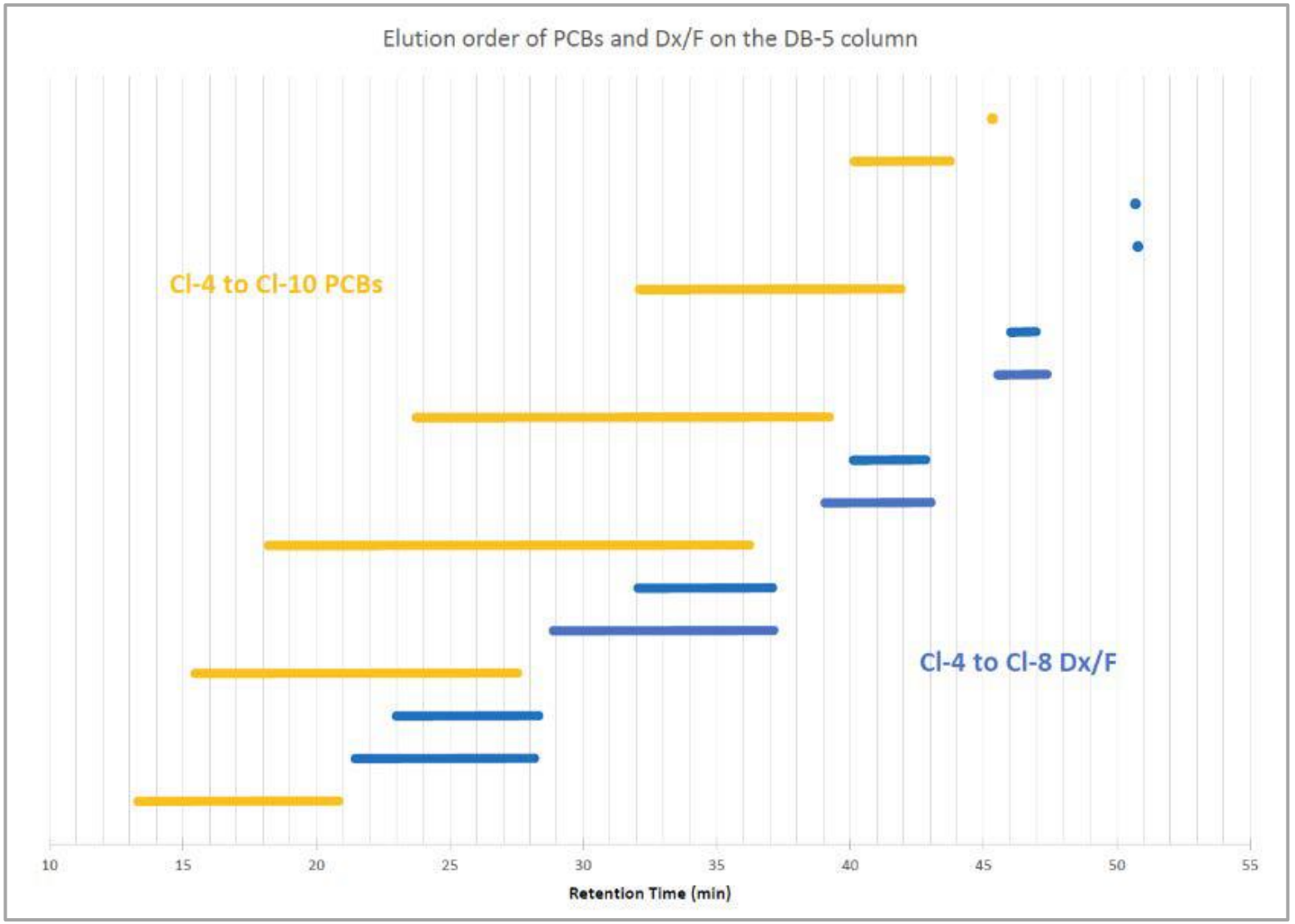


HEPTA AND OCTACHLORODIPHENYL ETHERS





DO PCBS INTERFERE?



FRAGMENTS OF HIGHER HOMOLOG PCBS

- High Concentrations of PCBs from higher levels of chlorination in extracts can give response in PCDD/PCDF channels
- Impact is minor, but if PCBs are not removed during extract cleanup then monitor MRM transitions for PCBs to detect them

Target	RT	Closely Eluting PCB	Observed Contribution
2,3,7,8-TCDF	25.32	PCB-141	0.17%
1,2,3,7,8-PeCDF	33.56	PCB-172 & PCB-192	0.06%
1,2,3,7,8-PeCDD	36.18	PCB-169	0.04%
1,2,3,4,6,7,8-HpCDF	45.54	PCB-209	0.05%

METHOD SUMMARY

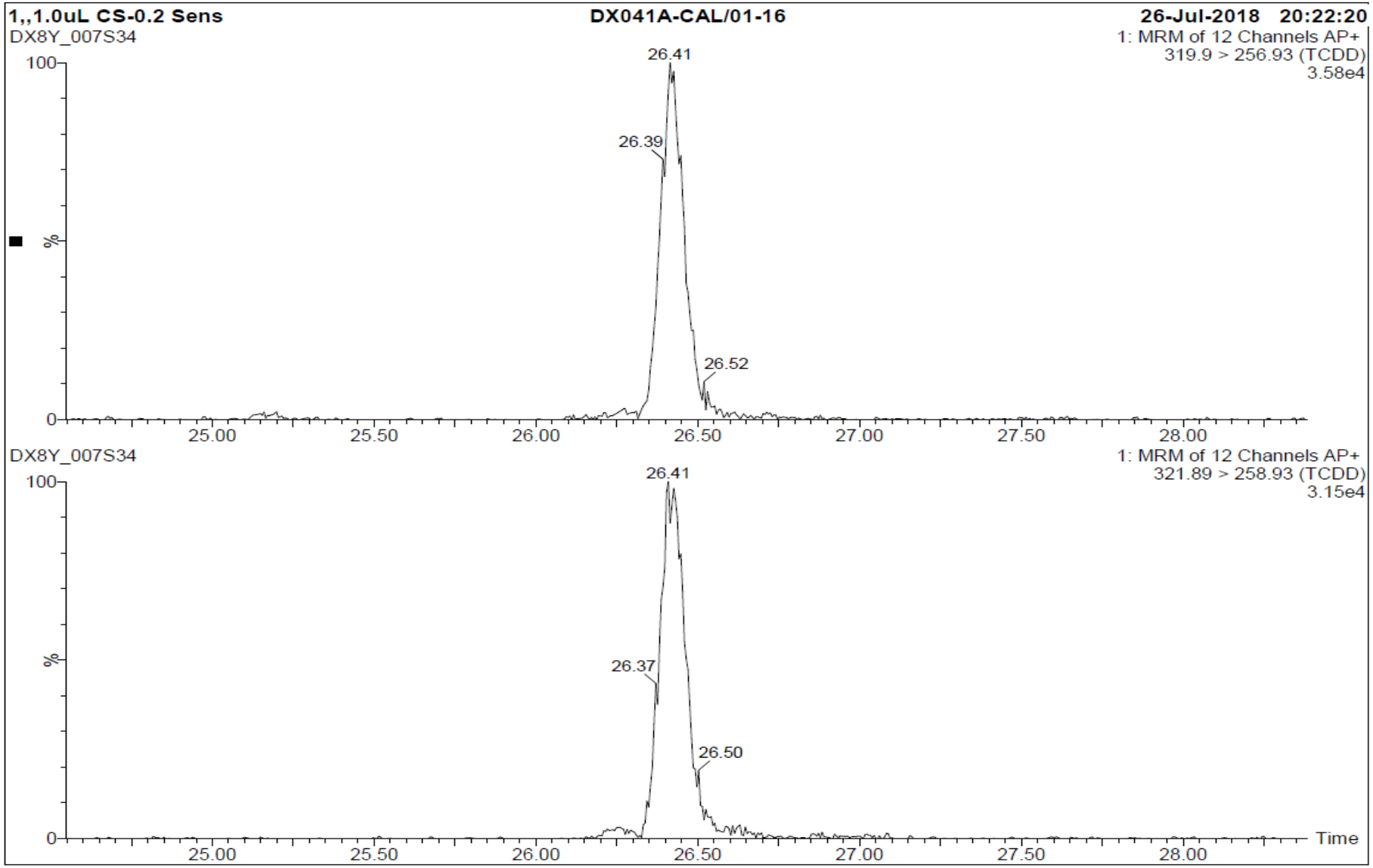
- 2 MRM transitions per analyte and labeled compound. Product ion ratios meet ±15% specifications as in 1613B.
- Two masses from molecular ion cluster as precursor masses. Product ions are from loss of [$CO^{35}Cl$].
- MRM transitions for chlorodiphenyl ethers are included
- Reference Compound is bled into the detector throughout the run and its MRM transition is monitored throughout the run
- PFTBA response replaces HRMS lock mass monitoring, same acceptance criterion.
- 12 hour mass resolution and accuracy check using reference compound scan replaces 12 hour 10,000 mass resolution verification

METHOD VALIDATION

DEMONSTRATE EQUIVALENCY TO METHOD 1613B. TIER 3 VALIDATION

- Sensitivity
- Linearity
- MDLs – 3 matrices
- IPRs – matrices
- PT and Reference Samples
- Robustness
    - Real world samples: 9 samples x 4 matrices
    - Comparison to HRMS results on same extracts

SENSITIVITY



- MRM Response of 2,3,7,8-TCDD for 0.1 pg injection

SENSITIVITY

	26-Jul-19	14-Aug-18	15-Aug-18	11-Sep-18	12-Sep-18	12-Dec-18
Compound	**S/N**	**S/N**	**S/N**	**S/N**	**S/N**	**S/N**
2,3,7,8-TCDF	456	798	1032	1463	1000	328
1,2,3,7,8-PeCDF	1056	1211	1158	1132	1213	466
2,3,4,7,8-PeCDF	1192	1432	1282	1234	1451	471
1,2,3,4,7,8-HxCDF	1519	738	942	723	2634	209
1,2,3,6,7,8-HxCDF	1491	753	937	710	2856	229
2,3,4,6,7,8-HxCDF	1739	797	970	812	2863	218
1,2,3,7,8,9-HxCDF	1496	704	941	741	2825	201
1,2,3,4,6,7,8-HpCDF	978	1647	1054	1364	1714	419
1,2,3,4,7,8,9-HpCDF	945	1583	940	1437	1666	393
OCDF	6267	3933	150507	300020	217422	2352
2,3,7,8-TCDD	359	198	420	8079	1688	285
1,2,3,7,8-PeCDD	471	959	1064	1206	991	659
1,2,3,4,7,8-HxCDD	1540	2153	1731	2888	1666	277
1,2,3,6,7,8-HxCDD	1495	1966	1696	2759	1585	289
1,2,3,7,8,9-HxCDD	1404	1905	1773	2659	1595	286
1,2,3,4,6,7,8-HpCDD	2951	4089	2454	9640	2849	594
OCDD	517	344	257	1142	521	253

- Signal to Noise Ratios for the 2,3,7,8-PCDD/PCDF in Multiple 1 µL Injections of the CS1 calibration Standard

LINEARITY

- %RSD of PCDD/PCDF Response Factors for Three Sets of Initial Calibration Data

Date acquired	26-Jul-18	12-Sep-18	12-Dec-18
Datafile ID	DX8Y_007	DX8Y_032	DX8Y_068B
Name	RRF %RSD	RRF %RSD	RRF %RSD
2,3,7,8-TCDF	3.7	2.8	5.7
1,2,3,7,8-PeCDF	3.5	3.3	5.4
2,3,4,7,8-PeCDF	3.0	2.3	4.5
1,2,3,4,7,8-HxCDF	3.8	3.3	5.7
1,2,3,6,7,8-HxCDF	3.9	2.2	4.8
2,3,4,6,7,8-HxCDF	3.5	2.7	5.0
1,2,3,7,8,9-HxCDF	3.8	2.5	12.8
1,2,3,4,6,7,8-HpCDF	3.1	2.6	6.9
1,2,3,4,7,8,9-HpCDF	3.2	2.4	15.4
OCDF	3.7	3.3	11.9
2,3,7,8-TCDD	3.2	3.3	5.2
1,2,3,7,8-PeCDD	2.5	2.3	5.6
1,2,3,4,7,8-HxCDD	3.1	2.6	4.8
1,2,3,6,7,8-HxCDD	2.9	3.3	4.5
1,2,3,7,8,9-HxCDD	3.6	2.3	8.8
1,2,3,4,6,7,8-HpCDD	2.8	3.2	13.9
OCDD	4.1	3.4	14.6
13C-2,3,7,8-TCDF	2.2	2.0	4.7

Date acquired	26-Jul-18	12-Sep-18	12-Dec-18
Datafile ID	DX8Y_007	DX8Y_032	DX8Y_068B
Name	RRF %RSD	RRF %RSD	RRF %RSD
13C-1,2,3,7,8-PeCDF	3.1	4.5	6.6
13C-2,3,4,7,8-PeCDF	2.7	4.0	7.7
13C-1,2,3,4,7,8-HxCDF	1.7	1.9	3.7
13C-1,2,3,6,7,8-HxCDF	1.6	1.7	3.7
13C-2,3,4,6,7,8-HxCDF	1.7	2.1	2.7
13C-1,2,3,7,8,9-HxCDF	2.4	1.7	3.3
13C-1,2,3,4,6,7,8-HpCDF	3.1	2.0	2.9
13C-1,2,3,4,7,8,9-HpCDF	3.6	3.6	4.0
13C-2,3,7,8-TCDD	1.6	2.0	4.7
13C-1,2,3,7,8-PeCDD	2.7	3.6	8.4
13C-1,2,3,4,7,8-HxCDD	1.5	1.7	3.4
13C-1,2,3,6,7,8-HxCDD	1.9	2.3	4.0
13C-1,2,3,4,6,7,8-HpCDD	3.6	3.2	2.1
13C-OCDD	3.8	5.2	4.4
13C-1,2,3,4-TCDD	14.8	12.7	10.6
13C-1,2,3,7,8,9-HxCDD	14.7	14.9	15.5
37Cl-2,3,7,8-TCDD	2.0	4.0	4.1

MDLS

Aqueous

	Initial MDL pg/L
2,3,7,8-TCDD	0.88
1,2,3,7,8-PECDD	1.01
1,2,3,4,7,8-HXCDD	1.12
1,2,3,6,7,8-HXCDD	1.06
1,2,3,7,8,9-HXCDD	0.98
1,2,3,4,6,7,8-HPCDD	0.92
OCDD	3.87
2,3,7,8-TCDF	0.56
1,2,3,7,8-PECDF	1.01
2,3,4,7,8-PECDF	0.94
1,2,3,4,7,8-HXCDF	1.10
1,2,3,6,7,8-HXCDF	0.95
1,2,3,7,8,9-HXCDF	1.09
2,3,4,6,7,8-HXCDF	0.96
1,2,3,4,6,7,8-HPCDF	0.99
1,2,3,4,7,8,9-HPCDF	0.89
OCDF	2.51

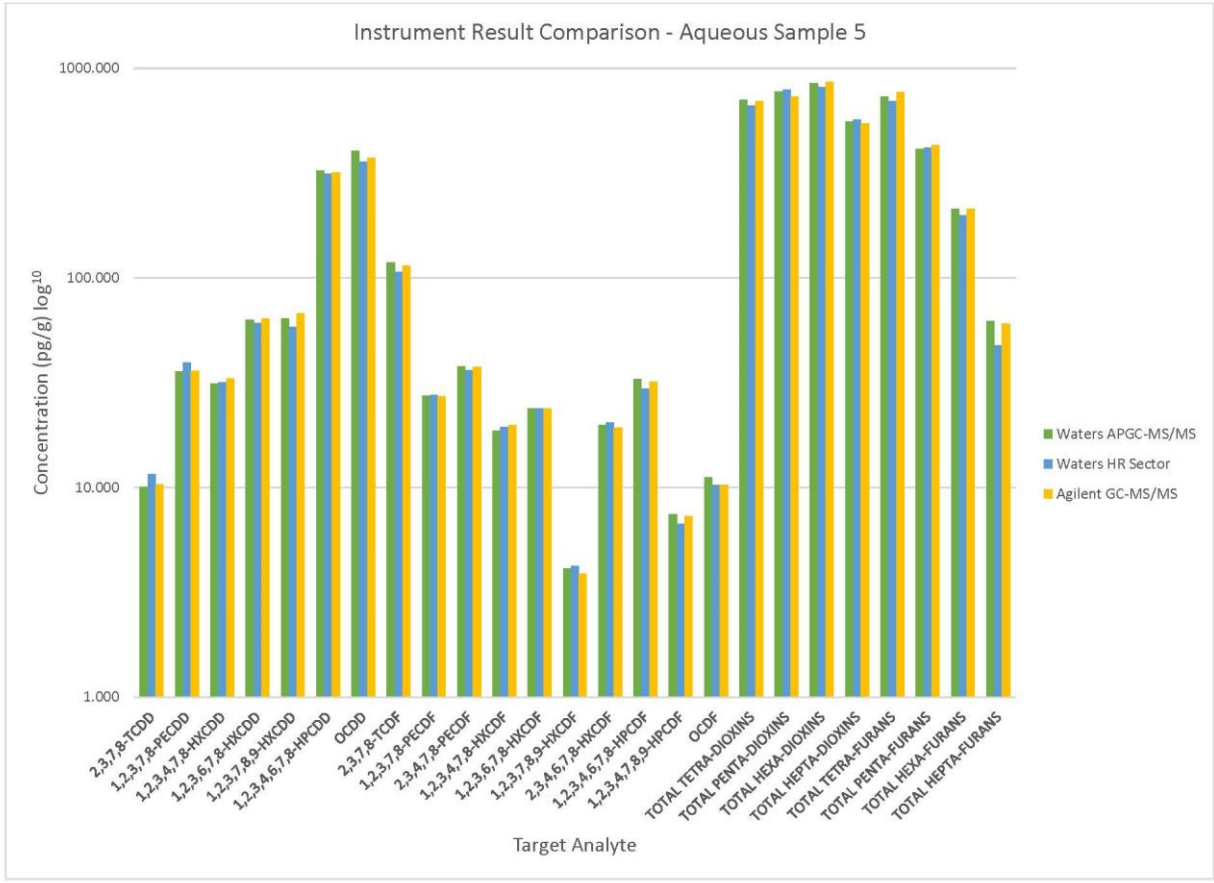
Solid

	Initial MDL pg/g
2,3,7,8-TCDD	0.029
1,2,3,7,8-PECDD	0.043
1,2,3,4,7,8-HXCDD	0.041
1,2,3,6,7,8-HXCDD	0.040
1,2,3,7,8,9-HXCDD	0.038
1,2,3,4,6,7,8-HPCDD	0.069
OCDD	0.341
2,3,7,8-TCDF	0.063
1,2,3,7,8-PECDF	0.038
2,3,4,7,8-PECDF	0.013
1,2,3,4,7,8-HXCDF	0.043
1,2,3,6,7,8-HXCDF	0.033
1,2,3,7,8,9-HXCDF	0.036
2,3,4,6,7,8-HXCDF	0.036
1,2,3,4,6,7,8-HPCDF	0.235
1,2,3,4,7,8,9-HPCDF	0.053
OCDF	0.345

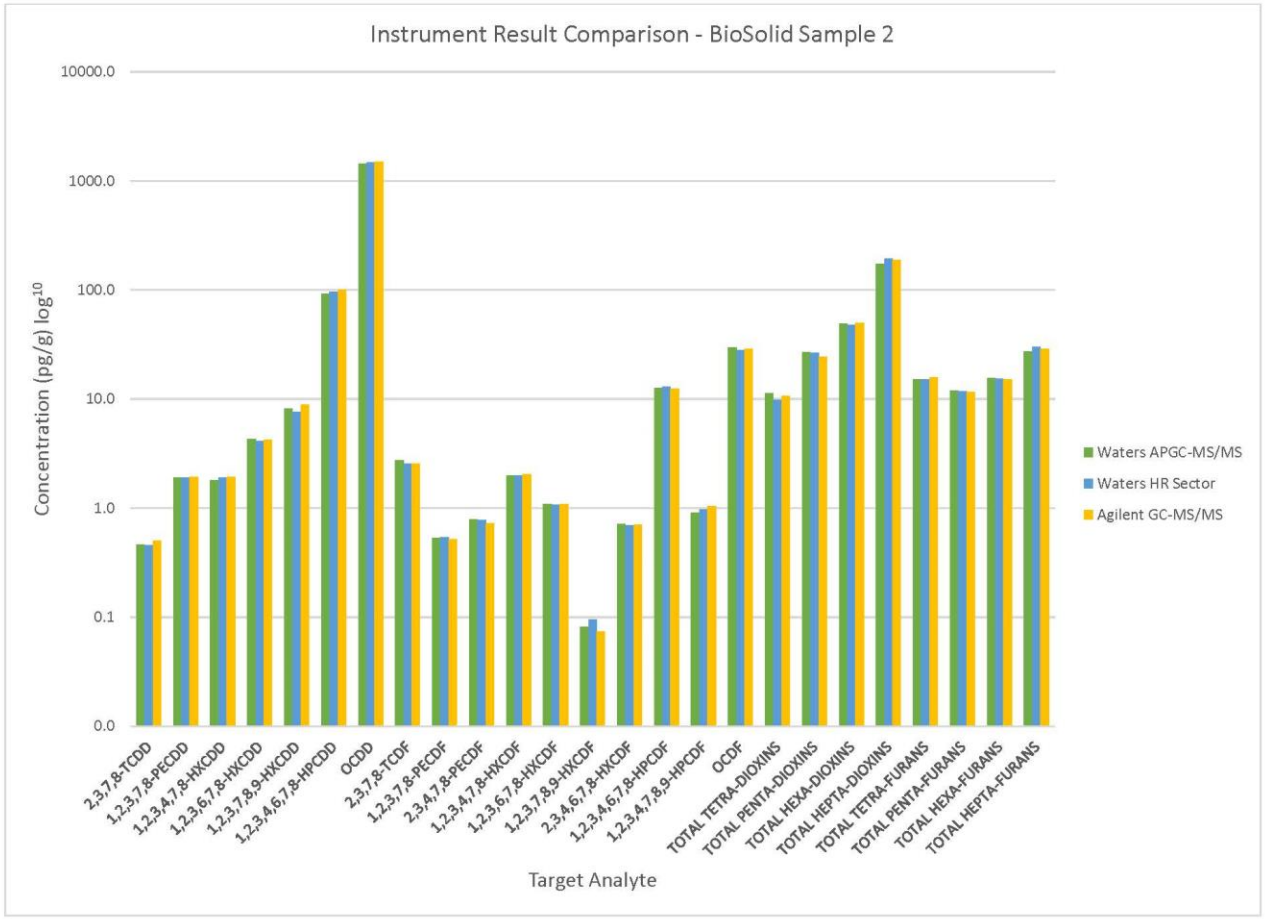
Tissue

	Initial MDL pg/g
2,3,7,8-TCDD	0.036
1,2,3,7,8-PECDD	0.032
1,2,3,4,7,8-HXCDD	0.042
1,2,3,6,7,8-HXCDD	0.040
1,2,3,7,8,9-HXCDD	0.041
1,2,3,4,6,7,8-HPCDD	0.055
OCDD	0.094
2,3,7,8-TCDF	0.054
1,2,3,7,8-PECDF	0.045
2,3,4,7,8-PECDF	0.035
1,2,3,4,7,8-HXCDF	0.028
1,2,3,6,7,8-HXCDF	0.036
1,2,3,7,8,9-HXCDF	0.048
2,3,4,6,7,8-HXCDF	0.041
1,2,3,4,6,7,8-HPCDF	0.058
1,2,3,4,7,8,9-HPCDF	0.055
OCDF	0.066

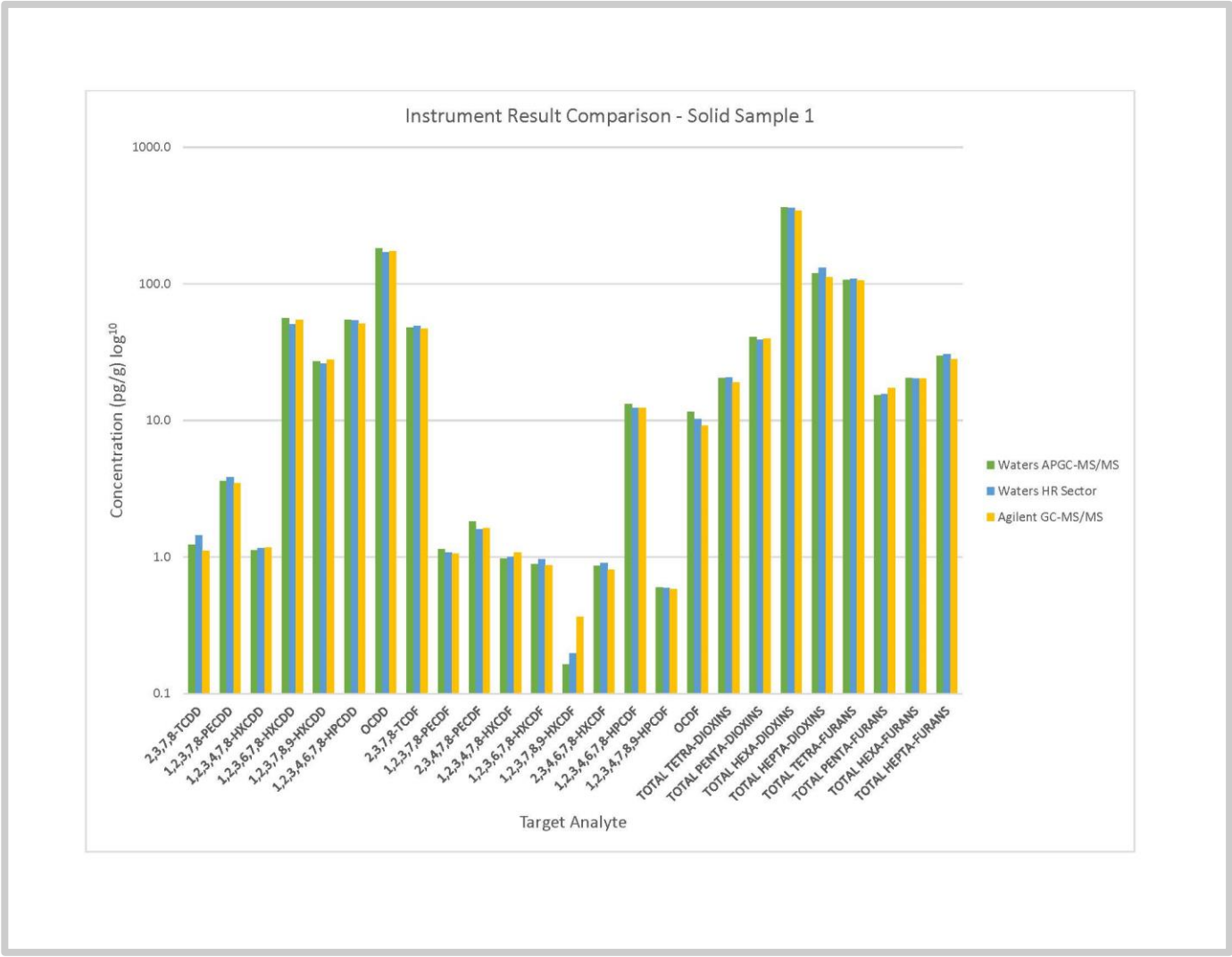
WASTEWATER SAMPLE MS/MS RESULTS VS. HRMS RESULTS



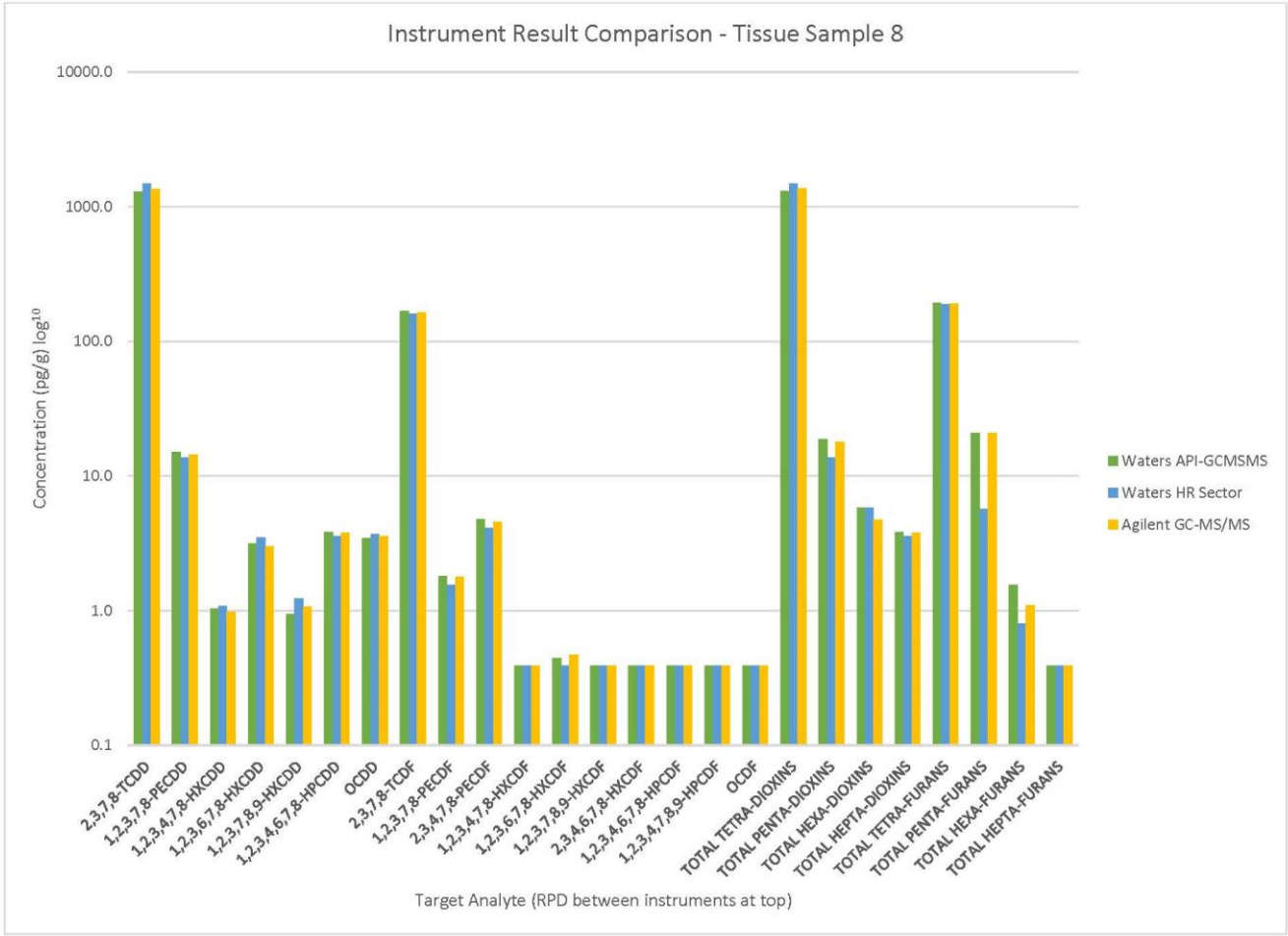
BIOSOLID SAMPLE



SEDIMENT SAMPLE
MS/MS RESULTS VS. HRMS RESULTS



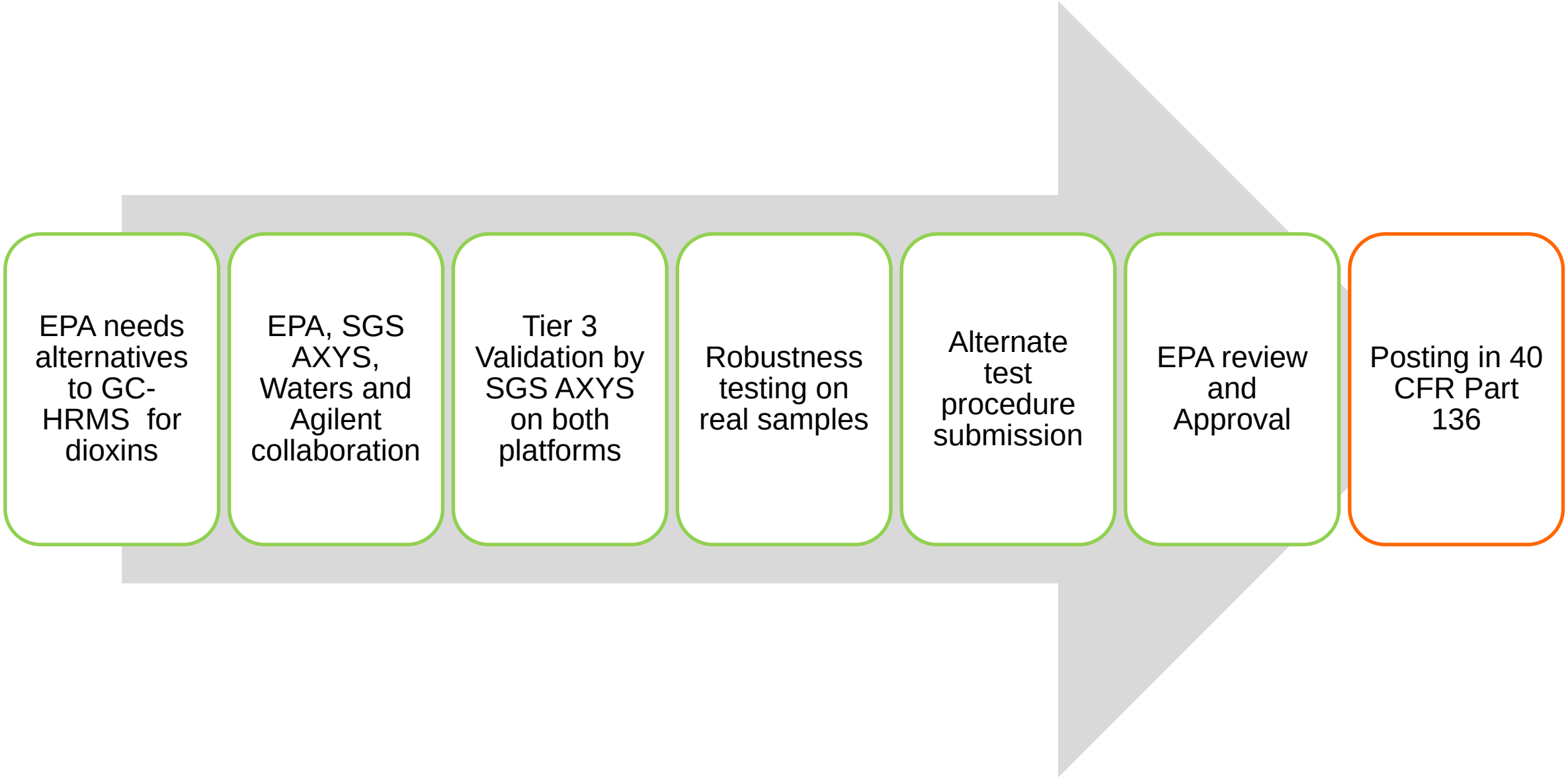
TISSUE SAMPLE



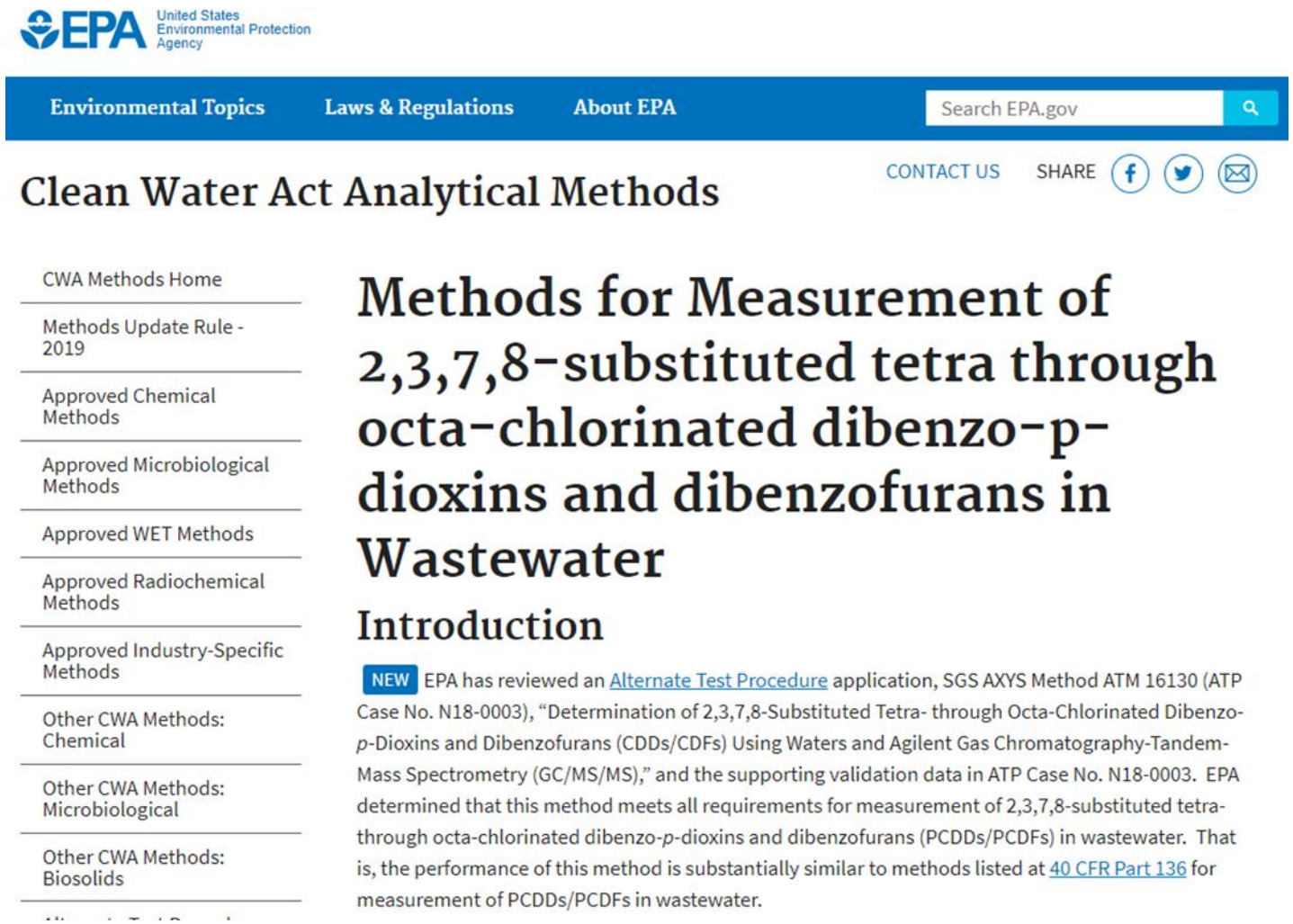
CONCLUSIONS

- A method for PCDD/PCDF by GC-MS/MS has been developed
- Method is fully validated on a Waters APGC with a Xevo TQ-XS MS system and on an Agilent GC with a 7010B Triple Quadrupole MS.
- Method meets all QC criteria of Method 1613B. HRMS-specific criteria and checks have been adapted to MS/MS
- ATP application has been approved
- SGS AXYS is accredited by CALA and NELAP for this method.
- Our clients are switching to MS/MS methods for Dioxins, PCBs and Pesticides.

SGS AXYS METHOD 16130



EPA APPROVAL STATUS



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- Other CWA Methods: Biosolids

Methods for Measurement of 2,3,7,8-substituted tetra through octa-chlorinated dibenzo-p-dioxins and dibenzofurans in Wastewater

Introduction

NEW EPA has reviewed an Alternate Test Procedure application, SGS AXYS Method ATM 16130 (ATP Case No. N18-0003), "Determination of 2,3,7,8-Substituted Tetra- through Octa-Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs) Using Waters and Agilent Gas Chromatography-Tandem-Mass Spectrometry (GC/MS/MS)," and the supporting validation data in ATP Case No. N18-0003. EPA determined that this method meets all requirements for measurement of 2,3,7,8-substituted tetra-through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs) in wastewater. That is, the performance of this method is substantially similar to methods listed at 40 CFR Part 136 for measurement of PCDDs/PCDFs in wastewater.

https://www.epa.gov/cwa-methods/methods-measurement-2378-substituted-tetra-through-octa-chlorinated-dibenzo-p-dioxins

ACKNOWLEDGEMENTS

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- SGS AXYS Senior Chemists: Xinhui Xiu, Robb Tones, Angie Schlak, Kristen Bowes

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