

WHITE PAPER

PAM-16130-SSI, a new Alternative Test Procedure (ATP) for the Determination of 2,3,7,8-Substituted Tetra- through Octa-Chlorinated Dibenzo-p-Dioxins and Dibenzofurans (CDDs/CDFs) Using Shimadzu Gas Chromatography Mass Spectrometry (GC-MS/MS)

INTRODUCTION

The US Environmental Protection Agency (EPA) has issued an Alternative Test Procedure Case Number N21-0001 as an alternative to EPA Method 1613B. This new method, PAM-16130-SSI, was submitted to EPA for review as an ATP for nationwide use under the Clean Water Act (CWA) ATP program. The method and supporting information, including the validation study report and method performance data, were reviewed and EPA determined that the method met all requirements for measurement of the regulated PCDDs/PCDFs in wastewater. As a result, this method is a candidate for inclusion in a future regulatory action in which EPA periodically updates the methods approved at 40 CFR Part 136 for use in CWA compliance monitoring. In the interim the method can be approved for National Pollutant Discharge Elimination System (NPDES) permits on a case-by-case basis as a limited-use ATP, as is outlined in the regulatory text at 40 CFR 136.5.

Dioxins and furans are the common names for two related families of environmentally persistent, toxic substances. These compounds include polychlorinated dibenzo dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs), which are unwanted by-products of industrial and natural processes, usually involving combustion. Dioxin and furan compounds vary greatly in their potential to cause adverse health effects. The most toxic of the chlorinated dioxin and furan molecules in laboratory animals is 2,3,7,8-tetachlorodibenzo-p-dioxin (TCDD).

EPA Method 1613 Revision B, EPA 621-B-94-005, for the analysis of polychlorinated dioxins and furan (PCDDs/PCDFs), is a gas chromatography with high-resolution mass spectrometry (GC/HRMS) procedure that identifies target analytes in the presence of other organic contaminants at very low picogram per liter (pg/L) concentrations. GC/HRMS instrumentation is more expensive than the more common low-resolution triple quadrupole instrumentation (GC-MS/MS), limiting the number of laboratories capable of running samples using Method 1613B. Approval of this GC-MS/MS method for measurement of PCDDs/PCDFs takes advantage of newer GC-MS/MS technology that lowers laboratory capital investment and operation cost, while not compromising method performance.

This ATP, Wastewater ATP Case Number N21-0001, is a limited modification of the instrumentation in Method 1613B and makes no changes to the existing extraction or cleanup procedures. Except for instrument-specific requirements (GC-MS/MS versus GC/HRMS), all quality control (QC) procedures and associated QC acceptance criteria in Method 1613B have been retained.

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Authors:
William C. Lipps, Yusuke Mizuno

Shimadzu Scientific Instruments,
7102 Riverwood Drive,
Columbia MD, 21046, USA

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Experimental

A Shimadzu GCMS-TQ8050 NX triple quadrupole GC-MS/MS (Figure 1) was equipped with a 60-meter DB-5MS UI capillary column and optimized to reproduce, to the extent possible, the conditions and chromatography used on the existing HRMS instrumentation, also equipped with a 60-meter DB-5MS UI column. Exact conditions were not used, allowing for slightly better separation on the GC-MS/MS; however, the total run time and retention times were not significantly different. Confirmation of 2,3,7,8- TCDF using a DB-225 column was not performed, as this was beyond the scope of the project. Analysts using the method would still have to perform the second column confirmation but would be allowed to use MS/MS detection. Figure 2 shows the GC conditions used for the validation.



Figure 1: Shimadzu GCMS-TQ8050 NX Triple Quadrupole Mass Spectrometer

- o Suggested GC operating conditions (modification allowed)
 - Injector temperature: 290 °C
 - Interface temperature: 300 °C
 - Initial temperature: 150 °C
 - Initial time: 0.5 minutes
 - Temperature program:
 - 150 to 220 °C, at 10 °C/minute
 - 220 °C for 20 minutes
 - 220 to 320°C, at 12 °C/minute
 - 320 °C for 10 minutes

Figure 2: GC Conditions used for the validation of PAM 16130-SSI

The GCMS-TQ8050 NX was equipped with a contaminant-resistant, highly sensitive and stable BEIS (Boosted Efficiency Ion Source) (Figure 3) that shields radiant heat generated by the filaments, ensuring that the ion source box temperature remains uniform. This shielding prevents active spots in the source and provides for high sensitivity and less contamination at lower box temperatures.

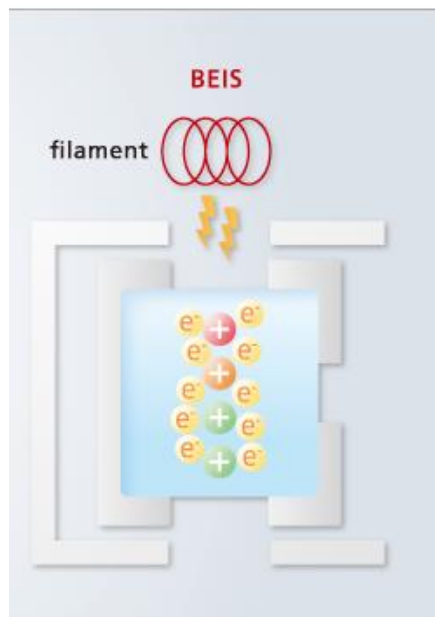


Figure 3: Shimadzu Highly Sensitive and Stable Boosted Efficiency Ion Source (BEIS)

The MRMs and collision energies for the PCDD/PCDFs were determined using the Shimadzu GCMS-TQ8050 NX MRM optimization tool, which automatically determines the transitions and collision energies in a single sequence, and then seamlessly incorporates them into the Shimadzu Smart Database series. At that point, the two most intense ions of each congener and internal standard were chosen. Using a flexible MS event, the Smart MRM function determines the best dwell and loop times for the two transitions in a single method, providing maximum precision and sensitivity. To improve the peak shape for 2,3,7,8-TCDD/TCDF at very low concentration levels, several event time settings were tested, and conditions chosen, that produced greater sensitivity for the target analytes and maintained adequate resolution between peaks. These time settings ensured a minimum of 15 integrations per peak. The final MRM settings and analyte retention times used in this validation are shown in Table 1.

Table 1: MRM Primary and Secondary Transitions with Approximate Retention Times

Analyte	Approximate RT (minutes)	Primary Transition	Secondary Transition
1,3,6,8-TCDF	26.156	303.90>240.90	305.90>242.90
1,3,6,8-TCDD	28.678	319.90>256.90	321.90>258.90
¹³ C ₁₂ -2,3,7,8-TCDF (EIS)	31.078	315.90>251.90	317.90>253.90
2,3,7,8-TCDF	31.092	303.90>240.90	305.90>242.90
¹³ C ₁₂ -1,2,3,4-TCDD (IIS)	31.251	331.90>268.00	333.90>270.00
1,2,3,4-TCDD	31.265	319.90>256.90	321.90>258.90
1,2,3,7/1,2,3,8-TCDD	31.527	319.90>256.90	321.90>258.90
1,2,3,9-TCDD	31.638	319.90>256.90	321.90>258.90
¹³ C ₁₂ -2,3,7,8-TCDD (EIS)	31.734	331.90>268.00	333.90>270.00
2,3,7,8-TCDD	31.746	319.90>256.90	321.90>258.90
³⁷ Cl ₄ -2,3,7,8-TCDD (cleanup)	31.747	327.90>262.90	325.90>260.90
1,2,8,9-TCDD	32.439	319.90>256.90	321.90>258.90
1,2,8,9-TCDF	32.554	303.90>240.90	305.90>242.90
HxCDDPE		375.80>305.90	
PFTBA		414.00>264.00	
1,3,4,6,8-PeCDD	32.513	339.90>276.90	337.90>274.90
1,2,4,7,9-PeCDD	33.672	355.90>292.90	353.90>290.90
¹³ C ₁₂ -1,2,3,7,8-PeCDF (EIS)	34.337	351.90>287.90	349.90>285.90
1,2,3,7,8-PeCDF	34.344	339.90>276.90	337.90>274.90
1,2,3,7,8-PeCDD	35.199	355.90>292.90	353.90>290.90
1,2,3,8,9-PeCDD	35.461	355.90>292.90	353.90>290.90
¹³ C ₁₂ -2,3,4,7,8-PeCDF	34.992	351.90>287.90	349.90>285.90
2,3,4,7,8-PeCDF	34.999	339.90>276.90	337.90>274.90
¹³ C ₁₂ -1,2,3,7,8-PeCDD	35.191	367.90>303.90	365.90>301.90
1,2,3,8,9-PeCDF	35.64	339.90>276.90	337.90>274.90
HpCDDPE		409.80>339.90	
PFTBA		414.00>264.00	
1,2,3,4,6,8-HxCDF	36.083	373.80>310.90	375.80>312.90
¹³ C ₁₂ -1,2,3,4,7,8-HxCDF (EIS)	36.900	385.80>321.90	387.80>323.90
1,2,3,4,7,8-HxCDF	36.906	373.80>310.90	375.80>312.90
1,2,4,6,7,9-HxCDD	36.452	389.80>326.90	391.80>328.90
¹³ C ₁₂ -1,2,3,6,7,8-HxCDF (EIS)	36.983	385.80>321.90	387.80>323.90
1,2,3,6,7,8-HxCDF	36.990	373.80>310.90	375.80>312.90
¹³ C ₁₂ -2,3,4,6,7,8-HxCDF (EIS)	37.373	385.80>321.90	387.80>323.90
¹³ C ₁₂ -1,2,3,4,7,8-HxCDD (EIS)	37.472	401.80>337.90	399.90>335.90
1,2,3,4,7,8-HxCDD	37.480	389.80>326.90	391.80>328.90
¹³ C ₁₂ -1,2,3,6,7,8-HxCDD (EIS)	37.541	401.80>337.90	399.90>335.90
1,2,3,6,7,8-HxCDD	37.549	389.80>326.90	391.80>328.90
¹³ C ₁₂ -1,2,3,7,8,9-HxCDD (IIS)	37.740	401.80>337.90	399.90>335.90
1,2,3,7,8,9-HxCDD	37.748	389.80>326.90	391.80>328.90
1,2,3,7,8,9-HxCDF	37.379	373.80>310.90	375.80>312.90
¹³ C ₁₂ -1,2,3,7,8,9-HxCDF (EIS)	38.002	385.80>321.90	387.80>323.90
2,3,4,6,7,8-HxCDF	38.008	373.80>310.90	375.80>312.90
OCDDPE		445.80>373.80	
PFTBA		414.00>264.00	
1,2,3,4,6,7,9-HpCDD	39.328	423.80>360.80	425.80>362.80
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDD (EIS)	39.964	435.80>371.80	437.80>373.80
1,2,3,4,6,7,8-HpCDD	39.971	423.80>360.80	425.80>362.80
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDF (EIS)	39.102	419.80>355.90	421.80>357.90
1,2,3,4,7,8,9-HpCDF	39.107	407.80>344.80	409.80>346.80
¹³ C ₁₂ -1,2,3,4,7,8,9-HpCDF (EIS)	40.444	419.80>355.90	421.80>357.90
1,2,3,4,7,8,9-HpCDF	40.450	407.80>344.80	409.80>346.80
NCDPE		479.70>407.80	
PFTBA		414.00>264.00	
¹³ C ₁₂ -OCDD (EIS)	42.703	469.80>405.80	471.80>407.80
OCDD	42.712	457.70>394.70	459.70>396.70
OCDF	42.930	441.80>378.80	443.80>380.80
DCDPE		513.70>443.70	
PFTBA		414.00>264.00	

Analyte-specific qualifier ion ratios were verified by the analysis of 29 standards over a period of two months. A mean and standard deviation were calculated to determine statistical control limits. In addition, the average mean of each chlorination level was compared and is summarized in Table 2, which is included in the method.

These ratios are used as a positive identification that detected peaks in unknown samples are a PCDD or PCDF isomer and are used for qualitative confirmation.

Table 2: Method Ion Abundance Ratios

Species Monitored	MRM Transition Precursor m/z (Primary/Secondary)	Transition Product Ion Ratio (%)	QC Limit (±15%)	
			Lower	Upper
Cl ₄ CDD	(M+2)/M	96	82	110
Cl ₄ CDF	(M+2)/M	96	82	110
Cl ₅ CDD	M/(M+2)	79	67	91
Cl ₅ CDF	M/(M+2)	79	67	91
Cl ₆ CDD	(M+4)/(M+2)	65	55	75
Cl ₆ CDF	(M+4)/(M+2)	64	54	74
Cl ₇ CDD	(M+4)/(M+2)	79	67	91
Cl ₇ CDF	(M+4)/(M+2)	80	68	92
Cl ₈ CDD	(M+4)/(M+2)	96	82	110
Cl ₈ CDF	(M+4)/(M+2)	94	80	108

■ Determination of Sensitivity

The Shimadzu GCMS-TQ8050 NX equipped with a BEIS produces little noise reaching the detector which, in turn, produces a very high signal-to-noise ratio (SNR). Additionally, the lower source temperature requirement of the Shimadzu GCMS-TQ8050 NX with a BEIS produces less fragmentation, providing abundant precursor ions and improving sensitivity. Long-term sensitivity was demonstrated by the analysis of five injections of the lowest calibration standard, CS-1, over a period of two months (Table 3).

These data demonstrate that the Shimadzu GCMS-TQ8050 NX readily met the minimum 10:1 SNR requirement of Method 1613B. An example chromatogram of 2,3,7,8-TCDD from the CS1 data collected on 6/29/2021 is shown in Figure 4.

Table 3: Signal-to-Noise Ratio of Six Separate 1-µL Injections of the CS-1 Standard Over Two Months

Name	4/26/2021 14:04	4/29/2021 16:25	6/2/2021 16:42	6/14/2021 12:09	6/29/2021 10:34
	CS-1	CS-1	CS-1	CS-1	CS-1
	S/N	S/N	S/N	S/N	S/N
2,3,7,8-TCDD	735.48	686.49	1443.46	994.3	294.76
1,2,3,7,8-PeCDD	3199.64	3415.7	2853.78	4999.41	1472.14
1,2,3,4,7,8-HxCDD	163.6	169.5	168.54	184.16	121.63
1,2,3,6,7,8-HxCDD	171	192.26	182.31	182.71	122.24
1,2,3,7,8,9-HxCDD	174.89	173.75	166.6	171.29	119.37
1,2,3,4,6,7,8-HpCDD	2872.66	3339.04	3603.14	4548.05	987.45
OCDD	6306.63	7664.08	8549.21	9057.53	1428.29
2,3,7,8-TCDF	1190.15	954.64	906.12	1898.67	357.22
1,2,3,7,8-PeCDF	3825.26	4078.94	1820.39	1820.3	1664.04
2,3,4,7,8-PeCDF	2197.68	2534.48	1356.94	1669.16	680.91
1,2,3,4,7,8-HxCDF	4202.14	5666.97	2902.16	4970.72	1993.94
1,2,3,6,7,8-HxCDF	4175.47	5486.35	2784.79	4709.98	1751.17
1,2,3,7,8,9-HxCDF	601.71	396.25	712.96	476.19	1166.67
2,3,4,6,7,8-HxCDF	764.57	520.81	776.68	556.61	1460.54
1,2,3,4,6,7,8-HpCDF	4988.73	4329.66	3204.9	4264.82	1097.58
1,2,3,4,7,8,9-HpCDF	3205.44	1498.29	936.58	1564.88	614.02
OCDF	5758.5	6684.66	4874.74	10173.63	1455.2

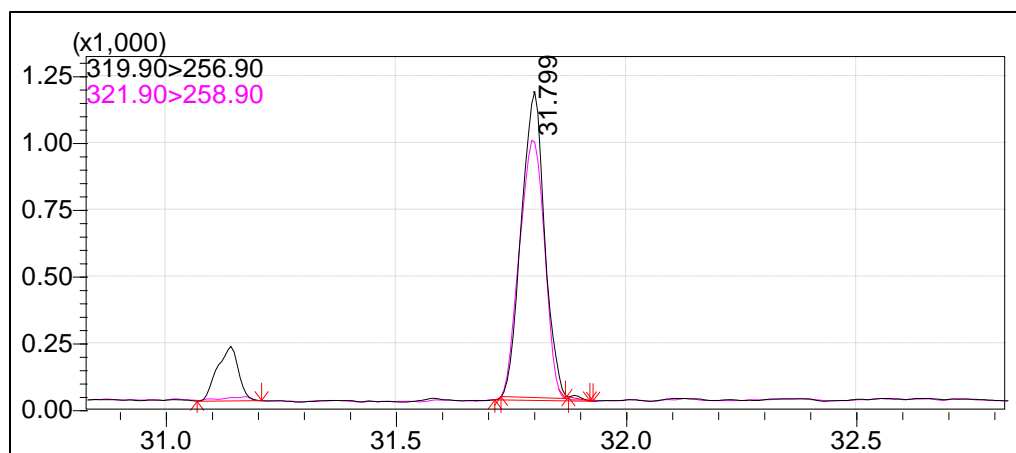


Figure 4: Chromatogram of 2,3,7,8-TCDD in the 6/29/2021 CS-1 standard.

■ Determination of Linearity

The Shimadzu GCMS-TQ8050 NX showed excellent linearity over the entire EPA Method 1613B calibration range. Initial calibration (ICAL) reports were prepared from five separate dates spanning two months. All Method 1613B specifications were met, including sensitivity (all analytes have SNR greater than 10:1 in CS1) and MRM transition product ion ratios within the $\pm 15\%$ limit.

Table 4 shows the Mean RF of each of these five separate calibrations collected on five separate days over a period of two months.

Table 4: Mean RF of Five Separate Calibrations Over a Period of Two Months

Parameter	210426 Mean RF	210429 Mean RF	210602 Mean RF	210614 Mean RF	210629 Mean RF
2,3,7,8-TCDD	1.214	1.192	1.205	1.193	1.178
2,3,7,8-TCDF	1.174	1.146	1.166	1.179	1.115
1,2,3,7,8-PeCDD	1.062	1.059	1.061	1.069	1.052
1,2,3,7,8-PeCDF	1.063	1.045	1.056	1.057	1.033
2,3,4,7,8-PeCDF	1.14	1.131	1.14	1.145	1.12
1,2,3,4,7,8-HxCDD	1.067	1.092	1.063	1.069	1.05
1,2,3,6,7,8-HxCDD	1.089	1.04	1.061	1.059	1.101
1,2,3,7,8,9-HxCDD	1.1	1.07	1.05	1.025	1.063
1,2,3,4,7,8-HxCDF	1.064	1.078	1.063	1.064	1.079
1,2,3,6,7,8-HxCDF	1.078	1.055	1.045	1.058	1.061
1,2,3,7,8,9-HxCDF	1.021	1.012	1.01	1.013	1.031
2,3,4,6,7,8-HxCDF	1.095	1.096	1.081	1.086	1.085
1,2,3,4,6,7,8-HpCDD	1.097	1.091	1.112	1.103	1.113
1,2,3,4,6,7,8-HpCDF	1.106	1.108	1.102	1.091	1.077
1,2,3,4,7,8,9-HpCDF	1.077	1.081	1.079	1.071	1.043
OCDD	1.123	1.12	1.084	1.081	1.044
OCDF	1.177	1.166	1.182	1.233	1.219
¹³ C ₁₂ -2,3,7,8-TCDD (EIS)	1.527	1.486	1.449	1.435	1.434
³⁷ Cl ₄ -2,3,7,8-TCDD	3.771	3.57	3.571	3.448	3.513
¹³ C ₁₂ -1,2,3,7,8-PeCDD (EIS)	1.44	1.389	1.375	1.389	1.204
¹³ C ₁₂ -1,2,3,4,7,8-HxCDD (EIS)	0.97	0.96	0.955	0.953	0.982
¹³ C ₁₂ -1,2,3,6,7,8-HxCDD (EIS)	0.927	0.949	0.949	0.971	0.945
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDD (EIS)	0.831	0.84	0.823	0.823	0.774
¹³ C ₁₂ -OCDD (EIS)	0.657	0.663	0.655	0.663	0.585
¹³ C ₁₂ -2,3,7,8-TCDF (EIS)	1.778	1.804	1.76	1.758	1.785
¹³ C ₁₂ -1,2,3,7,8-PeCDF (EIS)	1.761	1.729	1.765	1.818	1.509
¹³ C ₁₂ -2,3,4,7,8-PeCDF (EIS)	1.914	1.871	1.888	1.932	1.574
¹³ C ₁₂ -1,2,3,4,7,8-HxCDF (EIS)	1.214	1.217	1.197	1.213	1.214
¹³ C ₁₂ -1,2,3,6,7,8-HxCDF (EIS)	1.161	1.183	1.168	1.19	1.177
¹³ C ₁₂ -2,3,4,6,7,8-HxCDF- (EIS)	1.214	1.216	1.205	1.21	1.212
¹³ C ₁₂ -1,2,3,7,8,9-HxCDF- (EIS)	1.096	1.11	1.108	1.133	1.048
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDF (EIS)	1.016	1.045	1.04	1.066	0.946
¹³ C ₁₂ -1,2,3,4,7,8,9-HpCDF- (EIS)	0.993	1.006	0.991	1.026	0.929

■ Water Matrices

Table 5 shows water Ongoing Precision and Recovery (OPR) results along with the average recovery and % RSD which pass the Initial Precision and Recovery (IPR) limits and the OPR limits for all analytes.

Extracts of water samples previously extracted, analyzed, and reported by Method 1613B using GC-HRMS were obtained and analyzed using the Shimadzu GCMS-TQ8050 NX. Water samples were selected to cover nine specific industry categories (Table 5). Specific samples in this report are identified by a partial laboratory-assigned identification number (ID); however, this ID is intentionally incomplete and does not enable recognition of the source of samples or the identity of the customer.

The important point to note is that all real-world samples were submitted to the laboratory for PCDD or PCDF analysis.

Table 5: Water Sample Matrices Correlated with ID

Sample ID	Industry or Water Type
28001	Municipal wastewater from Tennessee (WWTP TN)
98001	Petroleum refinery
21001	Plastic manufacturing
64001	Health care products manufacture
68002	Window manufacturing
87001	Municipal wastewater from Ohio (WWTP OH)
70001	Pulp and paper industry
79001	Specialty chemicals and resins manufacturing
73001	Athletic field equipment manufacturing

Except for the WWTP -TN matrix, all water samples obtained acceptable recovery for each Extracted Internal Standard (EIS) (Table6). Although there is no explanation for the low recovery in the WWTP (TN) sample, we did compare recovery with the HRMS results on the same extract (Figure 3). The recoveries by both the Shimadzu GCMS-TQ8050 NX and HRMS detection are virtually equivalent. Therefore, the data in Table 6 and Figure 5 show that using a Shimadzu GCMS-TQ8050 NX as a replacement for GC-HRMS in Method 1613B obtains equivalent results.

Table 6: Percent recoveries of EIS Compounds in Nine Separate Water Matrices

EIS	21001	68002	28001	87001	70001	98001	64001	79001	73001
¹³ C ₁₂ -2,3,7,8-TCDD	65	70	29	68	61	75	72	77	64
¹³ C ₁₂ -1,2,3,7,8-PeCDD	89	76	23	79	64	85	79	80	79
¹³ C ₁₂ -1,2,3,4,7,8-HxCDD	80	79	17	68	49	82	90	78	65
¹³ C ₁₂ -1,2,3,6,7,8-HxCDD	78	73	19	71	49	90	72	82	61
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDD	85	79	17	80	49	92	81	88	60
¹³ C ₁₂ -OCDD	73	74	15	77	42	83	68	72	42
¹³ C ₁₂ -2,3,7,8-TCDF	67	72	31	72	66	81	69	80	66
¹³ C ₁₂ -1,2,3,7,8-PeCDF	77	72	23	69	60	76	74	73	72
¹³ C ₁₂ -2,3,4,7,8-PeCDF	83	72	24	73	62	80	71	75	73
¹³ C ₁₂ -1,2,3,4,7,8-HxCDF	79	77	19	71	49	84	71	81	63
¹³ C ₁₂ -1,2,3,6,7,8-HxCDF	84	82	20	72	53	90	80	86	69
¹³ C ₁₂ -2,3,4,6,7,8-HxCDF	83	80	19	77	54	88	73	84	67
¹³ C ₁₂ -1,2,3,7,8,9-HxCDF	109	84	22	97	76	101	94	110	69
¹³ C ₁₂ -1,2,3,4,6,7,8-HpCDF	83	79	17	74	52	91	75	84	62
¹³ C ₁₂ -1,2,3,4,7,8,9-HpCDF	80	78	17	75	49	89	74	83	61
³⁷ Cl ₄ -2,3,7,8-TCDD	75	82	80	82	83	77	74	86	92

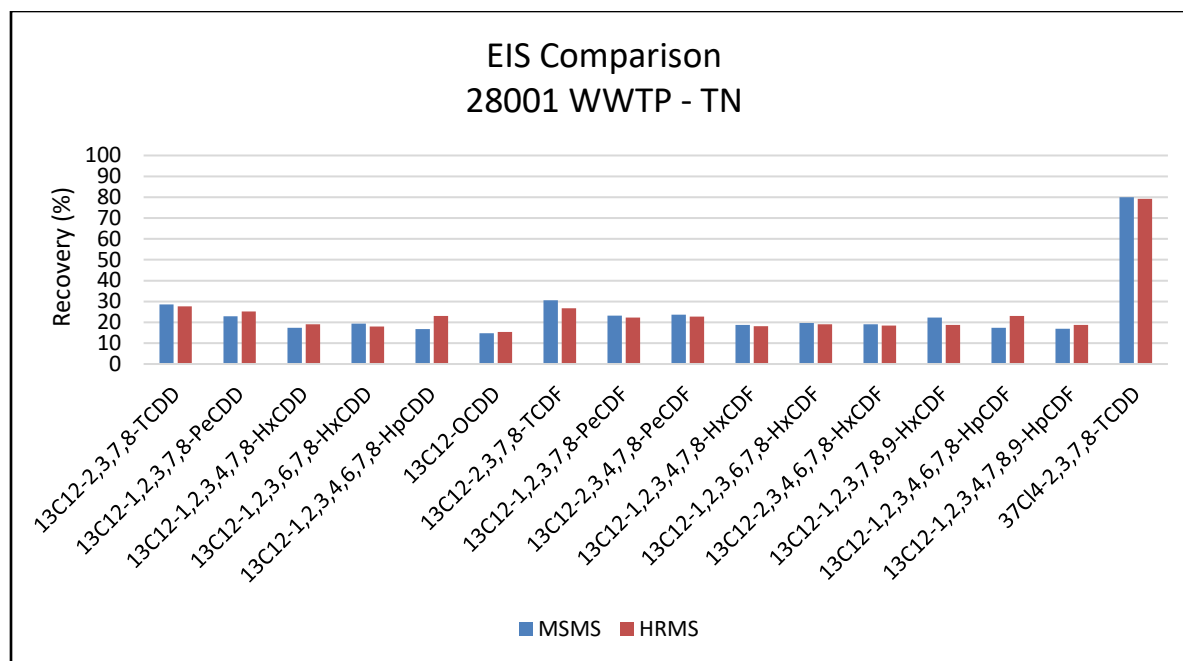


Figure 5: Comparison of MS/MS and HRMS EIS recovery on the WWTP TN sample.

Conclusion and Recommendations

This validation consists only of replacement of a GC-HRMS with a Shimadzu GCMS-TQ8050 NX for the analysis of PCDDs and PCDFs in wastewater matrices. Except for method conditions relating specifically to operation of the Shimadzu GCMS-TQ8050 NX, including instrument software calculations, all other aspects of the EPA-approved method remain the same. The validation laboratory, Pace Analytical Services in Minneapolis, Minnesota, is an accredited laboratory that analyzes PCDDs and PCDFs in water matrices by Method 1613B on a routine basis. Samples extracts that were analyzed by the production lab and reported to customers were selected and analyzed on the Shimadzu GCMS-TQ8050 NX. There were no modifications to the extraction procedure.

We made every effort to duplicate the chromatographic conditions of the production laboratory instrumentation to the extent feasible. Due to the differences in the gas chromatograph, there was some variability in the chromatography and injector and source temperatures.

Retention times and peak resolution of the Shimadzu GCMS-TQ8050 NX approximate that of the production lab and met Method 1613B requirements. The source temperature of the Shimadzu GCMS-TQ8050 NX instrument is lower; however, the lower temperature does not lead to contamination and results in better sensitivity due to a higher concentration of fragmented ions. These data were found to meet method acceptance criteria and results from the two measurements were generally equivalent.

The method and supporting information, including the validation study report and method performance data, were reviewed by EPA, and EPA determined that the method met all requirements for measurement of the regulated PCDDs/PCDFs in wastewater. As a result, this method is currently a candidate for inclusion in a future regulatory action. In the interim, PAM-16130-SSI can be approved as a limited-use ATP for NPDES permits on a case-by-case basis. Limited use approval is granted by the Regional ATP Coordinator, typically for a specific discharge or facility (and its laboratory).

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SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1222, Fax: 410-381-1222
URL: www.ssi.shimadzu.com

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