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Analysis of Dioxins Utilizing Time-of-Flight for Low Level Quantitation

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The World Health Organization categorizes dioxins as part of the “dirty dozen” of persistent organic pollutants. There are 210 congeners of dioxins, of these, the 17 with chlorine atoms in the 2,3,7,8 positions are classified as highly toxic and require trace level detection. In addition to the toxicity, they bioaccumulate in the fat tissue of animals, with a half-life of 7-11 years. The food chain is the primary source of the main human exposure. In 2008, several tons of pork in Ireland was recalled and destroyed due to a dioxin contamination 200x the safe limit. The current GC/MS method designated to the analysis of the environmental dioxin contamination is EPA 1613B¹, which requires specific chromatographic separation and high resolving power mass spectrometry for confident identifications.

The data presented in this poster illustrates the analytical capability of an accurate mass high resolution GC/Q-TOF for the analysis of dioxins. EPA method 1613b¹ has several data requirements to pass the criteria for a successful analysis of dioxins. The qualifying ion ratio cannot exceed 15% of theoretical, the resolving power must be >10,000 using the 10% valley calculation, the mass accuracy must be within 5ppm, and the lowest calibration standard (CS2) must have a S/N value >10. The 7250 GC/Q-TOF meets or exceeds each of these requirements.



Figure 1: Agilent 7250 GC/Q-TOF

Sample Preparation:

Chemical standards were provided by Accustandard for the 5-point calibration curve. 1613b prepped samples were provided by Eurofins TestAmerica (West Sacramento, CA) to evaluate the method with real-world extractions. Calibration curve and continuing calibration standards provided quantitative results for the extracted samples. Toluene and hexane were used for the autosampler solvent washes to reduce the amount of carry-over. Lockmass introduction is discussed below to meet the method criteria, but not necessary with this instrument because reduced mass assignment drift. Mass calibration between injections is a more productive approach for mass accuracy confidence. Analytical conditions for the GC/Q-TOF platform are listed in Table 1

Lockmass Introduction:

The Agilent Thermal Separation Probe installed into the multimode inlet(MMI) delivered a consistent amount of perfluorokerosene(PFK) into the ion source throughout the analysis (Figure 2). The MMI was kept at a constant temperature of 50 °C, using clean, dry air and connected to the MS with a 10m x 100µm deactivated tube which provided a flow of 0.05 mL min⁻¹.

Software:

All of the data analysis was performed with the MassHunter Suite. This included MassHunter Qualitative Analysis 10, and MassHunter Quantitative Analysis 10 using the SureMass peak detection algorithm.

Table 1: Agilent 7250 GC/Q-TOF; 7890B GC Parameters

GC and MS Conditions:	
Column	DB-5ms, 60 m, 0.25 mm ID, 0.25 µm film
Injection volume and liner	1 µL 2mm straight liner; UI
Pulsed Splitless	40psi for 1 min; 0.9min purge
Inlet temperature	290 °C
Oven temperature program	180 °C for 1 min 30 °C/min to 270 °C; hold 1min 2 °C/min to 310 °C 10 °C/min to 320 °C; hold 6min
Carrier gas	Helium; 1.0 mL min ⁻¹
Transfer line temperature	300 °C
Source temperature	280°C
Quadrupole temperature	150°C
Spectral range	225 to 650 m/z
Spectral acquisition rate	3 Hz, both centroid and profile
Electron Energy	70 eV
Emission	6µA

Results and Discussion

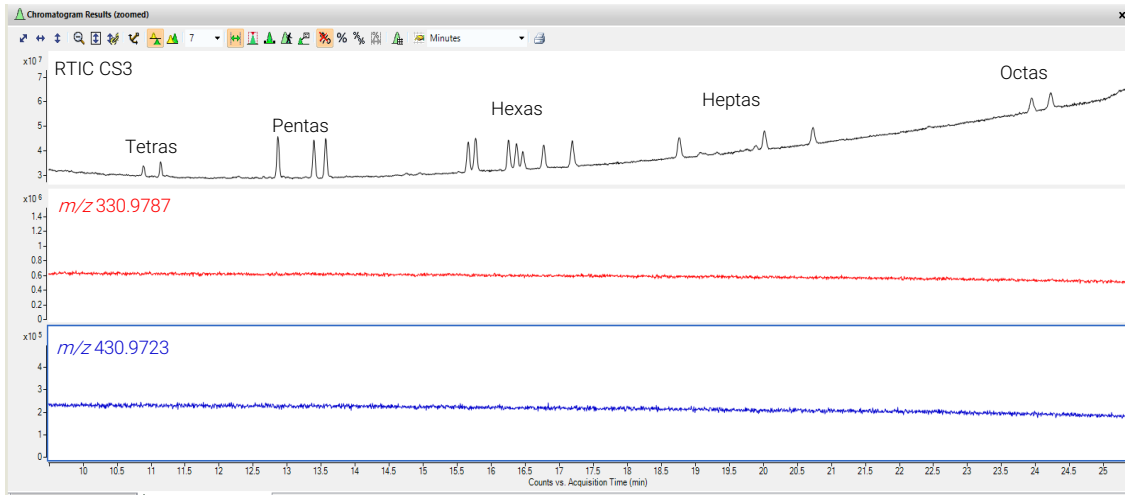


Figure 2: RTIC of CS3 with extracted Ion Chromatogram for two of the lockmass ions of PFK used for mass calibration.

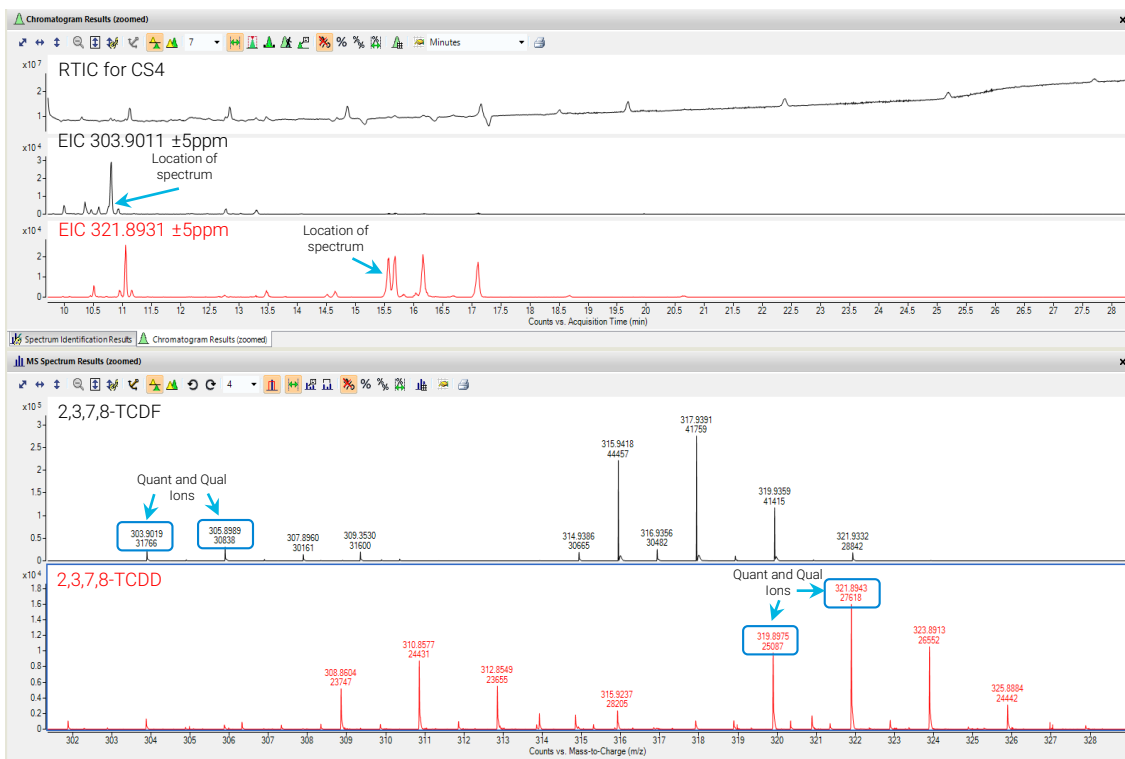


Figure 3: FWHM resolving power observed (bottom number) for the CS3, mid-point calibration standard for 2,3,7,8-TCDD and 2,3,7,8-TCDF.

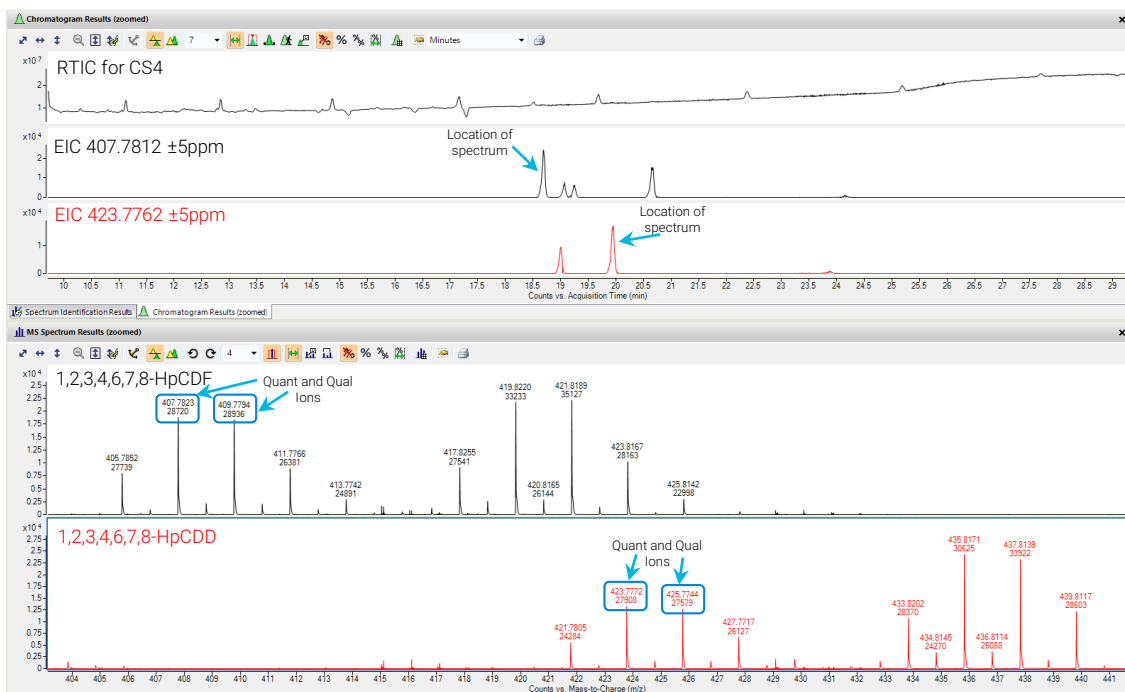


Figure 4: FWHM resolving power observed (bottom number) for the CS3, mid-point calibration standard for 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,6,7,8-HpCDD.

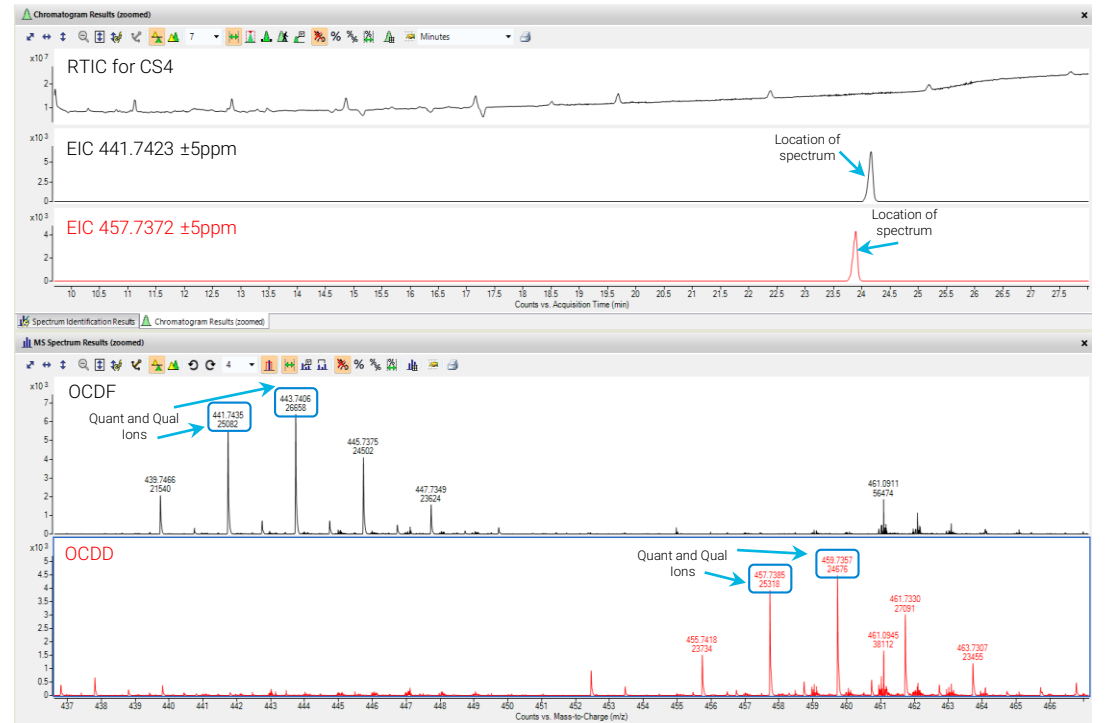


Figure 5: FWHM resolving power observed (bottom number) for the CS3, mid-point calibration standard for OCDF and OCDD.

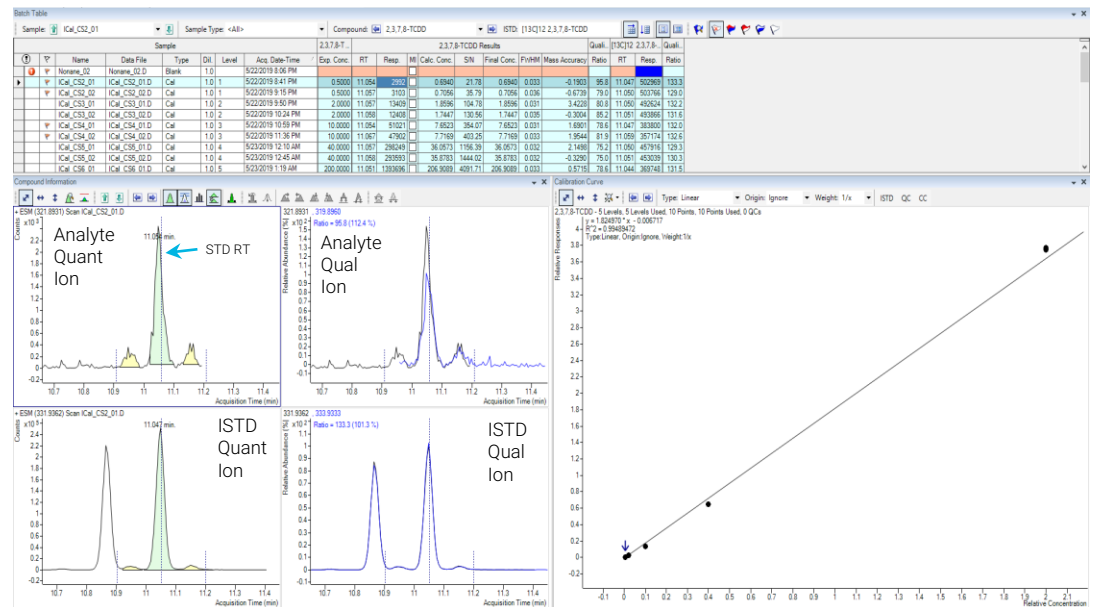


Figure 6: 2,3,7,8-TCDD lowest calibration quantitation with S/N, mass accuracy and ISTD response.

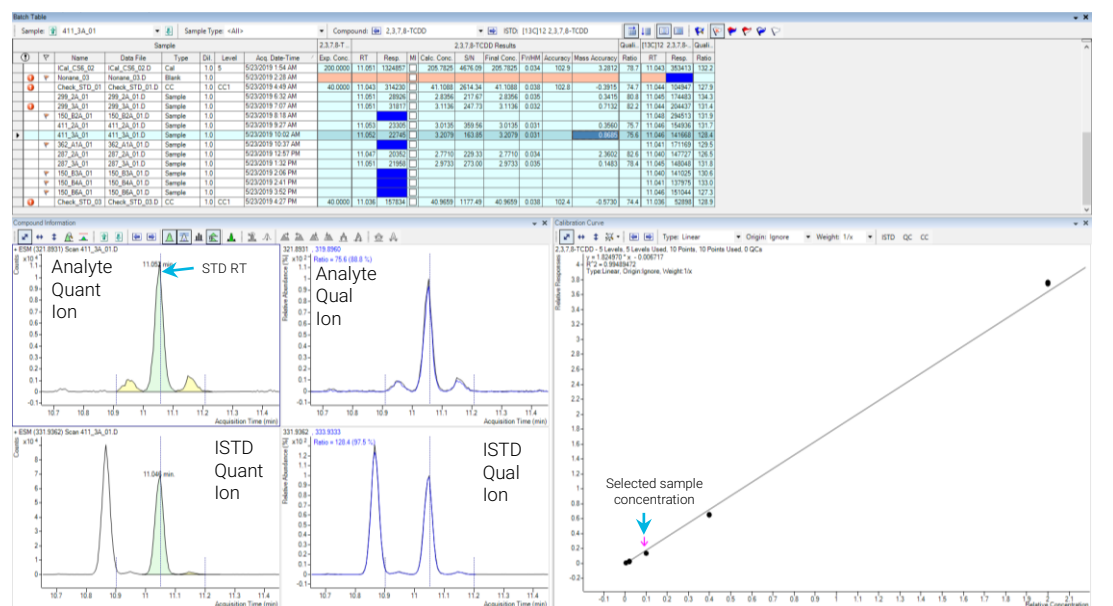


Figure 7: 2,3,7,8-TCDD calculated concentrations for several real-world extracted samples. CCs were ran 12-hours apart and provided calibration curve accuracy confidence.

Results and Discussion

Table 2: 20x dilution of CS5 was injected 8 times resulting in the following concentrations; 1ng mL⁻¹ TCDD & TCDF, 2.5ng mL⁻¹ Penta through Hepta, 10ng mL⁻¹ OCDD & OCDF. IDL was calculated using Student's t-test with a 99% confidence.

Compound Method	Rep_01	Rep_02	Rep_03	Rep_04	Rep_05	Rep_06	Rep_07	Rep_08				ng mL ⁻¹
Name	Area	Area	Area	Area	Area	Area	Area	Area	Average	STDev	RSD	IDL
1,2,3,4,6,7,8-HpCDD	12742	14565	13957	12710	11897	13086	12880	13675	13189.0	838.7	0.06	0.5
1,2,3,4,6,7,8-HpCDF	16479	22594	24337	23295	18331	24066	23362	24530	22124.3	3019.8	0.12	0.9
1,2,3,4,7,8,9-HpCDF	12627	18167	17554	15538	13577	15434	15401	16030	15541.0	1834.9	0.11	0.9
1,2,3,4,7,8-HxCDD	19719	23996	21407	21367	17502	19018	19558	19948	20314.4	1946.0	0.10	0.7
1,2,3,4,7,8-HxCDF	29872	36075	35077	32388	26850	31292	32669	32081	32038.0	2883.9	0.09	0.7
1,2,3,6,7,8-HxCDD	19020	24943	20586	20511	17693	22928	22717	20818	21152.0	2309.2	0.11	0.8
1,2,3,6,7,8-HxCDF	32614	36963	33059	33171	30183	32834	34837	34134	33474.4	1953.4	0.06	0.4
1,2,3,7,8,9-HxCDD	18437	22717	21028	19945	18239	19798	20398	19801	20045.4	1424.7	0.07	0.5
1,2,3,7,8,9-HxCDF	19897	24228	24604	22751	18558	21059	21556	24186	22104.9	2215.3	0.09	0.7
1,2,3,7,8-PeCDD	25121	29071	27178	26650	23121	25881	26830	26840	26336.5	1724.0	0.06	0.5
1,2,3,7,8-PeCDF	38142	46071	42357	41075	36847	41165	43002	43448	41513.4	2949.5	0.07	0.5
2,3,4,6,7,8-HxCDF	28740	34708	32366	29751	25829	29509	30520	32064	30435.9	2674.3	0.08	0.6
2,3,4,7,8-PeCDF	40203	44270	39925	41165	35841	39991	38045	43575	40376.9	2736.0	0.06	0.5
2,3,7,8-TCDD	6038	7842	8042	6614	5666	6767	5629	6705	6662.9	907.2	0.14	0.4
2,3,7,8-TCDF	6685	8292	6805	6973	6715	6983	7115	7806	7171.8	575.2	0.07	0.2
OCDD	14844	18766	18196	15281	12669	17232	17223	17485	16462.0	2033.1	0.12	3.5
OCDF	16030	22067	20710	17163	15176	19232	19661	21236	18909.4	2523.4	0.12	3.6

Table 3: Signal to noise for each analyte with respect to the calibration concentration. The lowest calibration standard is highlighted in blue to show each analyte is >10.

Compound Method	CS2		CS3		CS4		CS5		CS6	
Name	S/N	Resp.	S/N	Resp.	S/N	Resp.	S/N	Resp.	S/N	Resp.
1,2,3,4,6,7,8-HpCDD	644.54	303229	264.75	260546	315.22	183432	346.74	205249	592.8	219234
1,2,3,4,6,7,8-HpCDF	535.06	214919	689.23	190678	251.15	137157	299.78	159165	417	157652
1,2,3,4,7,8,9-HpCDF	161.44	163451	362.06	138510	251.66	93991	247.95	104984	271.98	107817
1,2,3,4,7,8-HxCDD	557.77	405837	485.88	363188	487.34	269649	429.28	300079	311.46	303614
1,2,3,4,7,8-HxCDF	650.44	371447	508.24	341398	551.27	257123	556.9	280229	680	274086
1,2,3,6,7,8-HxCDD	490.39	405837	395.18	363188	370.91	269649	429.28	300079	386.35	303614
1,2,3,6,7,8-HxCDF	650.44	371447	429.38	341398	551.27	257123	556.9	280229	627.1	274086
1,2,3,7,8,9-HxCDD	544.18	449604	447.78	407700	48.32	279405	448.23	317813	40.06	324780
1,2,3,7,8,9-HxCDF	412.85	252947	428.05	239139	294.34	158896	355.44	177271	181.21	180705
1,2,3,7,8-PeCDD	1103.74	592545	1117.96	543791	732.06	392381	900.8	441348	1204.9	441402
1,2,3,7,8-PeCDF	1597.76	939554	1490.02	873399	1075.49	655344	409.03	708179	1367.44	705442
2,3,4,6,7,8-HxCDF	625.31	345507	613.38	318097	431.46	228667	603.55	252140	617.17	253215
2,3,4,7,8-PeCDF	1539.43	940325	1083.29	847683	977.79	613773	1195.89	679789	1056.12	690386
OCDD	531.43	357078	423.91	280076	362.39	177791	438.15	206948	526.33	218232
1,2,3,7,8-TCDD	1037.59	569601	626.58	516957	905.26	429298	770.52	456612	786.03	447944
1,2,3,7,8-TCDF	982.06	538853	597.54	494540	814.1	381822	663.81	409987	860.71	401762
1,2,3,7,8-TCDF	1761.94	838685	1014.73	786886	1086.92	573289	1246.15	635143	1303.45	626658
1,2,3,7,8-TCDF	16.12	6128	74.31	20761	319.95	88690	723.98	417377	3684.07	2424298
1,2,3,4,6,7,8-HpCDD	30.38	8342	113.1	25388	190.05	89154	508.57	448096	3162.48	2743066
1,2,3,4,6,7,8-HpCDF	49.82	11917	86.37	42207	288.07	160362	1140.15	863318	1009.44	4999904
1,2,3,4,7,8,9-HpCDF	18.06	7752	75.59	30986	204.97	102956	522.91	512227	2702.56	3216108
1,2,3,4,7,8-HxCDD	41.37	9544	72.82	36680	102.93	136063	635.01	711841	2305.15	4165972
1,2,3,4,7,8-HxCDF	32.39	16622	135.28	59161	477.9	237228	1336.66	1251005	5078.75	7396482
1,2,3,6,7,8-HxCDD	41.37	9544	72.82	36680	230.63	136063	755.42	711841	2305.15	4165972
1,2,3,6,7,8-HxCDF	32.39	16622	135.28	59161	477.9	237228	1390.2	1251005	5078.75	7396482
1,2,3,7,8,9-HxCDD	12.81	12237	72.85	36292	245.53	136074	681.62	696725	2306.57	4168375
1,2,3,7,8,9-HxCDF	47.41	10570	122.89	43991	307.94	152201	978.04	788726	2875.38	4720151
1,2,3,7,8-PeCDD	35.98	12852	127.07	46936	427.96	186588	1339.36	969781	6920.23	5471838
1,2,3,7,8-PeCDF	74.9	20449	253.63	74792	472.11	302353	609.53	1517045	6443.85	9012186
2,3,4,6,7,8-HxCDF	84.87	14033	163.71	56051	390.07	210199	1054.57	1138956	4484.87	6711514
2,3,4,7,8-PeCDF	111.14	20032	223.94	70797	481.46	286077	1909.46	1473084	6437.96	8759891
2,3,7,8-TCDD	19.03	2927	83.43	11761	119.69	45676	1270.22	214743	1692.75	1227079
2,3,7,8-TCDF	20.23	3944	49.28	12398	170.45	52924	492.41	239347	347.06	1369294
OCDD	40.6	9557	26.1	25392	188.95	94465	732.05	474488	4756.47	3071322
OCDF	53.83	11633	79.6	34519	294.4	111974	789.57	592775	2937.81	3762547

For the qualifying ratios, an outlier is configured in MassHunter Quantitative analysis for each compound to flag if the sample has a deviation greater than 15%, the largest difference was 10% for the 10x dilution of the CS2 lowest calibration standard. The resolving power requirement of 10,000 by 10% valley converts to a requirement of >20,000 for FWHM (Figure 3). The data collects illustrated that the instrument was able to provide the required resolving power with the lowest calculation of ~24,000 and most were >32,000. The largest mass accuracy deviation observed was 2.4 ppm for the lowest calibration level while most were 1ppm or below. Eight replicate injections were performed with a diluted standard with varied concentrations of the different species. For the Tetra compounds the concentration was 1ng mL⁻¹, the penta- through hepta- was 2.5 ng mL⁻¹ and the octas were 10ng mL⁻¹ provided an instrument detection limit of 0.2-0.4, 0.4-0.9 and 3.5-3.6, respectively (Table 2). For the S/N requirement of the lowest calibration standard, the lowest value was 12, for one compound and the largest was 111 and an average of 44 (Table 3). Contaminated samples were also analyzed to calculate the amount of 2,3,7,8-isomer dioxins were present (Figure 7).

Conclusions

High resolving power, accurate mass and high sensitivity provided data to meet the performance criteria for EPA method 1613b.

- Time-of-flight spectral acquisition speeds provided accurate isotope intensities
- SureMass peak detection produced consistent mass accuracy
- Resolving power for the analytes exceeded the requirement of the method
- High resolving power quantitation provided excellent results for complex samples.

Reference

¹Telliard, W.A., 1994. Method 1613b Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. U.S. Environmental Protection Agency, Office of Water.