Meeting the Challenges of EPA 1613b Using Gas Chromatography with High Resolution Time-of-Flight Mass Spectrometry

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Comprehensive Analysis, High Resolution Mass Spectrometry, Time-of Flight MS, Dioxins, Persistent Organic Pollutants, Polyaromatic Hydrocarbons

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and furans (Figure 1) are some of the most toxic materials known to man. These persistent organic pollutants (POPs) have been linked to cancer and adverse reproductive and endocrine effects.[1,2] The "Gold Standard" for dioxin analysis is EPA 1613b Protocol which has rigid requirements for sensitivity, isotopic abundance, and resolving power ($R_{10\%}$ = 10,000). Some of the specific performance criteria are provided in Table 1. This has been historically achieved, and the method developed, exclusively using magnetic sector instruments.[3,4] In this analysis these instruments have operated in selected ion monitoring mode using slow scans in a targeted analysis. Other high performance systems including FTICR, orbitraps, and early GC-TOF systems failed to achieve one or more of the needed performance metrics. High resolution time-of-flight mass spectrometry provides for the acquisition of spectral data across a wide mass range, with the requisite resolving power ($R_{10\%}$ > 10,000), high mass accuracy, and appropriate sensitivity. This application note shows that the performance of the LECO Pegasus® GC-HRT meets or exceeds the requirements for dioxin analysis per the US EPA 1613b Protocol.

PCDF $C_{12}H_{8-(m+n)}OCl_{(m+n)}$

PCDD $C_{12}H_{8-(m+n)}O_2Cl_{(m+n)}$

Figure 1. General structures and formulas for polychlorinated benzofurans (PCDFs) and benzofurans (PCDDs).

Table 1. Performance Criteria of EPA Method 1613.

Method Attribute	Requirement
MS Resolving Power	Not Less Than 10,000 at 10% Valley at m/z 304
Ion Abundance Ratios	Not more than 15% Variance
Lock Mass	PFK
Lock Mass Stability	Not more than 20% variance
Sensitivity	0.5 ppb (minimally)
Ionization Conditions	28-40 eV

2. Results

Resolving Power

Resolving power is one of the attributes which has allowed magnetic sectors to perform this analysis effectively for the past three decades. Gas chromatography TOFMS systems have fallen short in this attribute historically. The implementation of FFP™ technology has provided the capability to achieve in excess of 20,000 (FWHM) resolving power at m/z 304. To demonstrate this, a spectrum from the analysis of TCDF is shown in Figure 2. The resolving power obtained is in high resolution mode with a factor of 2 additional resolving power available in ultra high resolution mode.

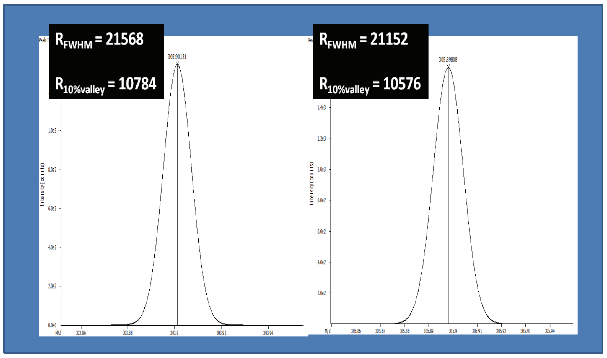


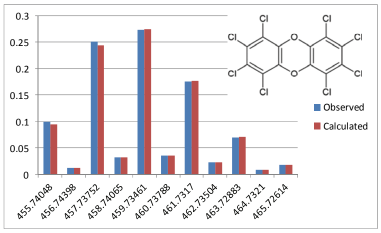
Figure 2. Mass Spectrum from the analysis of TCDF (m/z = 303.9016 and 305.8987) in high resolution mode. Shows the M and M+2 isotopes and has calculated resolutions of 10784 and 10576 (10% valley).

Relative Isotope Abundance (Ion Abundance Ratios)

The key to the confirmation of purity and identity of the analytes of interest is the relative isotope abundance (or Ion Abundance Ratios). The observed and theoretical relative isotope abundance values are provided for OCDD and OCDF in Figure 3. EPA 1613b stipulates that for a Dioxin having 8 chlorines the ratio of the (M+2) and (M+4) isotopes should be between 0.76 and 1.02 (averaged at 0.89). Examining the values provided, analysis on the Pegasus GC-HRT provides ratios of 0.916 and 0.910 for OCDD and OCDF, respectively. Similar values are observed for other polyhalogenated species.

OCDD

Mass	*Observed*	*Calculated*
455.74048	0.099	0.095
456.74398	0.012	0.012
457.73752	0.251	0.244
458.74065	0.032	0.032
459.73461	0.274	0.275
460.73788	0.036	0.036
461.73170	0.176	0.177
462.73504	0.022	0.023
463.72883	0.070	0.072
464.73210	0.009	0.009
465.72614	0.018	0.019



OCDF

Mass	*Observed*	*Calculated*
439.74541	0.101	0.095
440.74860	0.013	0.012
441.74251	0.252	0.245
442.74595	0.033	0.032
443.73966	0.277	0.275
444.74278	0.035	0.036
445.73659	0.173	0.177
446.73988	0.022	0.023
447.73368	0.068	0.071
448.73726	0.009	0.009
449.73088	0.017	0.018

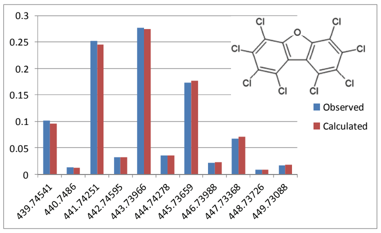


Figure 3. Molecular ion region for OCDD (Top) and OCDF (Bottom) showing good correlation between calculated and observed relative isotopic abundances.

Lock Mass Stability

EPA 1613b requires that the lock mass, internal calibration ions, maintain a constant and stable intensity throughout. Variations in the intensity are suggested to be the result of potential interfering compounds and may affect the mass accuracy or intensity of the analytes of interest. As such, the lock mass intensity must maintain at ±20%. To demonstrate this capability the stability of three ions from the PFTBA leak are tracked across the time window during which most dioxin compounds elute (800 until 1900 seconds). This is depicted in Figure 4 for specified ions from the PFTBA calibrant, total sample signal, and selected dioxins signal. The calibrant signals show an inherent signal stability of far less than 5% across the time window of interest for both 218.98 and 413.98 ions. EPA 1613b specifies perfluorokerosene (PFK) as the lock agent, but it is reasonable to assume that its behavior will be similar to that of PFTBA.

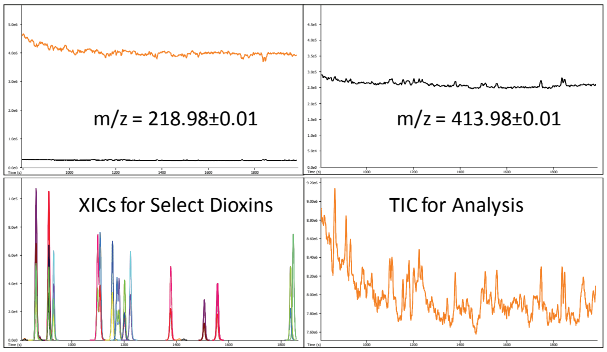


Figure 4. Signal stability of calibrant ions from PFTBA. Shown are signals from 218.98 and 413.98 from PFTBA. The retention of dioxin analytes of interest are provided (lower left) as is the total ion chromatogram (TIC) for the injection.

Sensitivity

Pivotal to the analysis of dioxins is the sensitivity of the analysis. The toxicity of these compounds mandates that extremely low levels be detectable. Even with enrichment the concentrations required are less than 1 ppb (ng/mL). The sensitivity of sectors is attributed to their ability to dwell on signal from single analytes. In contrast, TOFMS systems do not need to perform these selected ion monitoring analyses. Full spectral acquisition can be achieved on the Pegasus GC-HRT and sensitivities needed by EPA 1613b can be achieved.

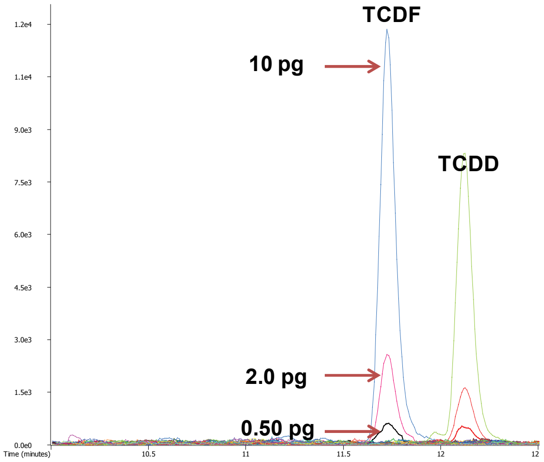


Figure 5. XIC of TCDF & TCDD CS1-CS3.

An overlay of the peaks generated by plotting the appropriate extracted ions for the tetra-substituted congeners (TCDF and TCDD) in standards CS1-CS3 is displayed in Figure 3. The lowest level analyzed, 0.5 pg on column, represents the minimal detectable amount in 1613b (Table 2 in Method 1613) yet provides a readily detectable peak in these analyses. Other minimum values are at or greater than 2.5 pg on column.

The dynamic range of the Pegasus HRT is illustrated by the linearity of area versus concentration plots for 2,3,7,8-TCDF and 2,3,7,8-TCDD from CS1 (0.50 pg/μL) to CS5 (200 pg/μL) standards (Figure 6). This is not the limit of the system but is a representation of capabilities and provides evidence of the ability to distinguish dioxin concentrations across a range appropriate for most analyses.

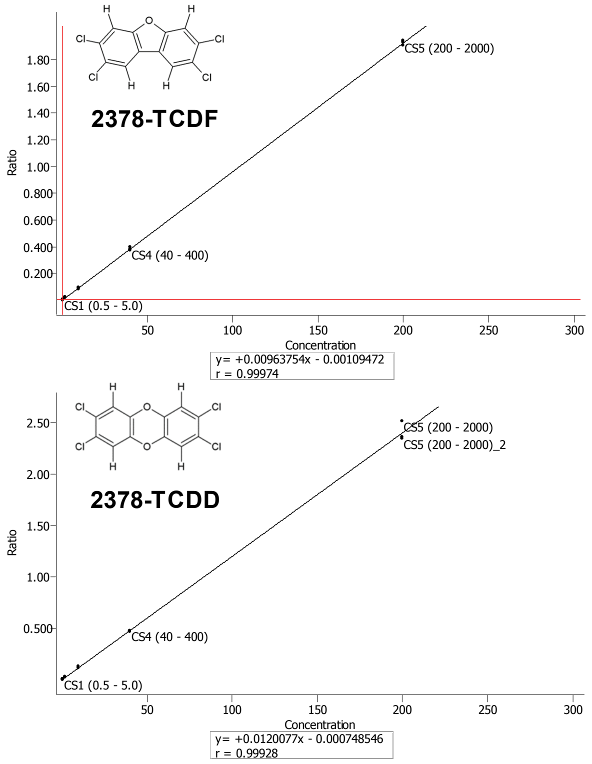


Figure 6. Calibration curves (CS1-CS5) for 2,3,7,8-TCDF (Top) and 2,3,7,8-TCDD (Bottom).

Ionization Energy

The ionization energy recommended in EPA 1613b is 28-40 eV, which is lower than the traditional 70 eV used in EI. This is done to provide for the maximum molecular ion intensity without the compromise of fragmentation. The Pegasus GC-HRT provides its optimal molecular ion intensity at the high end of this range (40 eV). Analysis on the Pegasus GC-HRT falls into this window with spectra from OCDD and OCDF (Figure 7) provided as examples. These show strong molecular ions and isotopes and provided matches of 811 and 808 (of 1000) for each of the two analytes.

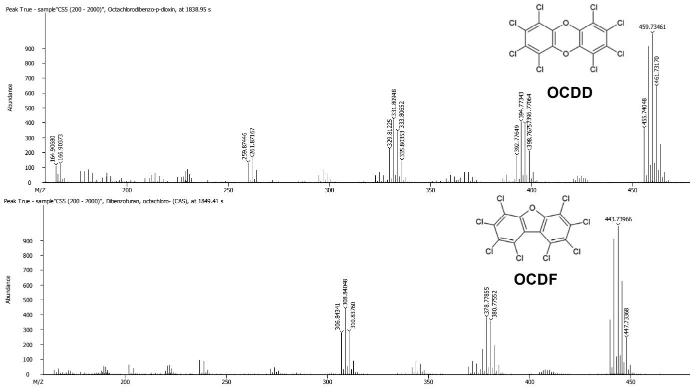


Figure 7. Peak true spectra for OCDD (A) and OCDF (B).

3. Conclusion

A point-by-point comparison of the requirements of EPA 1613b Protocol versus the observed performance on the Pegasus GC-HRT is provided. Table 2 provides a summary of the attributes and what was seen on the instrument.

This summary provides representative data to support the determination of dioxins and related compounds using high resolution time-of-flight MS as implemented on the Pegasus GC-HRT.

Table 2. Observed attributes of the Pegasus GC-HRT in meeting the criteria of EPA 1613b.

Method Attribute	Requirement	Pegasus GC-HRT
MS Resolving Power	Not Less Than 10,000 at 10% Valley at m/z 304	>10,000 for m/z 303.9016 and 305.8987
Ion Abundance Ratios	85-115% of Theory (1613b Section 10.2.2)	103%
Lock Mass Stability	Not more than 20% variance	< 5%
Sensitivity	0.5 ppb (minimally)	< 0.5 ppb
Ionization Conditions	28-40 eV	40 eV

4. References

[1] Kogevinas M, *Human Reproduction Update*, **2001**, *7(3)*, 331-339.

[2] Baccarelli A, Mocarelli P, Paterson DG, Bonzini M, Pesatori AC, Caporaso N, and Landi MT, *Environmental Health Perspectives*, **2002**, *110(12)*, 1169-1173.

[3] Schecter A, Birnbaum L, Ryan JJ, Constable JD, *Environmental Research*, **2006**, *101*, 419-428.

[4] Reiner EJ, *Mass Spectrometry Reviews*, **2010**, *29(4)*, 526-559.

5. Samples

Calibration standards (CS1 – CS5) containing native and isotopically labeled dioxins and furans were purchased from Wellington Laboratories (Guelph, Canada). Concentrations of the most toxic tetrachlorinated species ranged from 0.5 to 200 ng/mL. All concentrations are listed in Supplemental Tables 1 and 2 at the end of this note.

6. Experimental

Operational settings for the LECO Pegasus® GC-HRT

GC:	Agilent 7890
Column Type:	Restek Rxi-5Sil MS (30 m, 0.25 mm ID, 0.25 mm df)
Inj. Temp.:	250°C
Injection:	Splitless, 2 μL

Oven:

120°C(1)→220°C (20°C/min)→240°C (2.0°C/min)→ 250°C (1.0°C/min)→260°C (5.0°C/min)→265°C (1.0°C/min)

Carrier Gas:	He, 1.0 mL/min constant flow
MS:	Pegasus GC-HRT
Source Temp.:	250 °C
EI:	40 eV
Mass Range:	160 - 510 m/z High Resolution Mode