Quantification of Dioxins by GC-Orbitrap MS.

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Introduction:

The need for stringently high chromatographic and mass spectrometric resolution has long been recognized for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). These requirements are due to the extremely small concentrations of PCDD/Fs found in the environment and the need to regulate these chemicals at pg/g concentrations. Technologies to provide the required sensitivity and specificity for PCDD/F analysis were developed in the mid to late 1980s with the development of analytical protocols based on magnetic sector instrumentation (US-EPA 1997. Recent developments in high resolution Orbitrap technology make possible new levels of mass resolution and sensitivity suitable for identification and quantification of PCDD/Fs. This study compared results from Orbitrap GC/MS analysis of PCDD/Fs to data produced by using of a magnetic sector system and to the requirements of US-EPA methods for the analysis of PCDD/Fs.

Methods:

Analyses were carried out on a Q Exactive GC Orbitrap mass spectrometer interfaced to a Trace 1310 GC and a TriPlus RSH injector system (Thermo Scientific, Missisauga, Canada). Helium was used as the carrier gas and the TRACE GC was run in programmed flow mode with 0.5 ml/min help for 2 min, ramped to 1 ml min at 30 min and held at 1 ml/min for the duration of the run. GC oven temperature was ramped from 110 to 310 ˚C. PCDD/F standards were obtained from Wellington Laboratories (Guelph, ON, Canada).

To assess potential matrix effects on analyses of PCDD/F an extract of used motor oil was prepared using standard dioxin analysis procedures. This approach was chosen in preference to a PCDD/F spiking study to avoid possible problems with PCDD/F extraction efficiencies in this difficult matrix since the aim of the study was to assess matrix effects on MS analysis rather than on extraction and clean up procedures. This approach also allowed preparation of extracts with various weight equivalents of matrix extractables. Used motor oil (1 g) was dissolved in 200 ml hexane and washed with 5 aliquots of 25 ml of concentrated sulphuric acid. After the last acid wash the sample was washed 3 times with 25 ml aliquots of DI water. The hexane fraction was then decanted through anhydrous sodium sulphate before being concentrated to approximately 5 ml by rotary evaporation. The concentrated extract was then passed sequentially through three 10 g columns of silica gel before again being concentrated to 5 ml. The extract was next passed through a 10 g column of sulphuric acid silica gel (40% w/w). The extract was re-concentrated and then eluted through a 10 g alumina column by use of 50% dichloromethane in hexane. Volume of the final extract was 1 ml.

Extracts of fish tissue were prepared using previously described methods (Wan et al. 2010). Identification and quantification of PCDD/Fs and dioxin-like PCBs were performed using a Hewlett-Packard 5890 series high-resolution gas chromatograph interfaced with a Micromass® Autospec® high-resolution mass spectrometer (HRGC–HRMS) (Micromass®, Beverly, MD). A split/splitless injector was used in splitless mode. Chromatographic separation was achieved on a DB-5MS fused silica capillary column (60 m length, 0.25 mm ID, 0.1 μm film thickness, Agilent, CA).

Samples were extracted and analysed by magnetic sector GC/MS in 2014 and remaining extracts were archived at -20 °C until analysis in the current study.

The Orbitrap system consisted of a Q-Exactive GC Orbitrap interfaced to a Trace 1310 gas chromatograph (GC) and a TriPlus RSH injector system (Thermo Scientific, Missisauga, ON). The GC was equipped with a split/splitless injector module and injections were of 1ul in splitless mode. The analytical column was a 60 m 0.25 mm diameter DB5ms column with a 0.1 micron film thickness (Agilent, CA), helium was the carrier gas. GC conditions were set to optimize separation of 2,3,7,8- TCDD and TCDF from their respective interfering congeners using the TDTFWD standard (Wellington Laboratories, Guelph, ON). The transfer line temperature was 285 °C and the source temperature was 250 °C. The mass spectrometer was operated in scan mode at a resolution of 60,000, while collecting full profile data from 300-550 m/z. The AGC target was $1x10^6$ ions per scan. Data processing and calibration were carried out using Xcalibur 4.0 (Thermo Scientific, Missisauga, ON). For calibration all US-EPA method 1613 were used except that 2 confirmation ions not 1 were used for identification of each congener and full profile not SIM data were collected. A time window and resolution check solution (TDTFWD, Wellington Laboratories, Guelph, ON) was used to confirm retention times for the first eluting TCDF and last eluting octa-congener but time windows were not used as full profile acquisition (m/z 300-550) was used for the duration of the run. Due to the inherent mass accuracy and stability of the Orbitrap detector lock masses were not used to confirm mass accuracy however a 2 mmu identification criterion was used for all quantitation and confirmation ions.

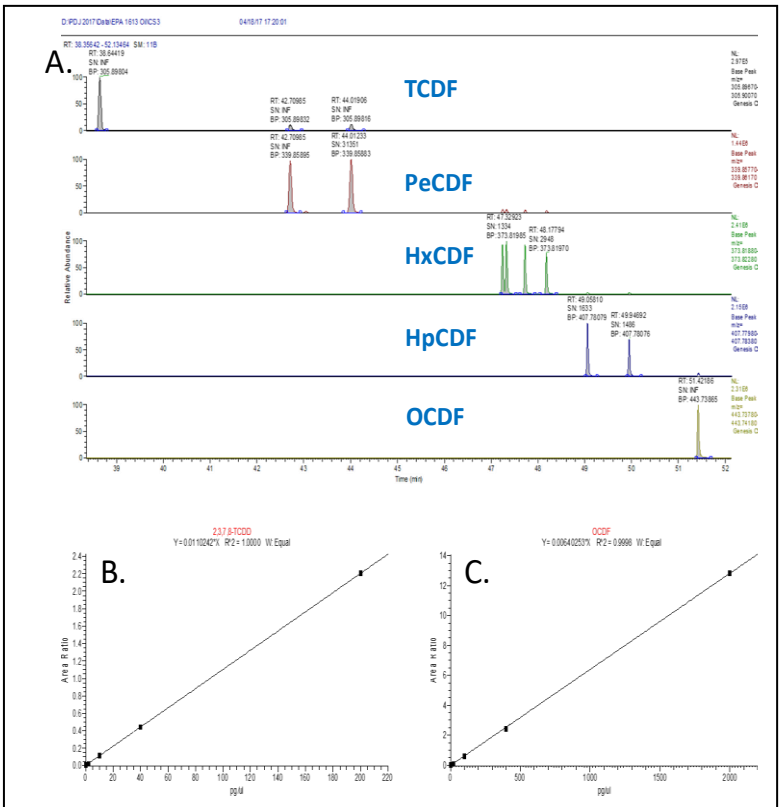

Figure 1. (A) Chromatograms of all native dibenzofuran congeners in the method 1613 CS3 standard. **(B, C)** Seven point calibration curves for 2,3,7,8-TCDD and OCDF for the method 1613 standards. Standards include the 5 initial calibration standards plus 2 ongoing calibration verification CS3 injections.

Results:

Sensitivity of the Orbitrap system for detection of PCDD/F congeners was demonstrated by analysis of US EPA Method 1613 calibration standards (Figure 1). The Orbitrap MS system was able to detect all PCDD/F congeners of interest with high sensitivity. For the 2 calibration curves prepared during this study only one congener calibration curve had a coefficient of determination of less than 1.000 (OCDF 0.9998), these curves included all the initial

calibration standards as well as the CS3 mid and end run calibration verification injections runs so 'n's were between 7 and 8. For all congeners two confirmation ions were used (c.f method 1613 using only one confirmation ion) and observed/predicted variations for the standard concentrations were <10%. The system was also able to detect and quantify all compounds of interest in the "CSL" standard which represents a 5x dilution of the CS1 standard (data not shown) demonstrating a limit of detection of for 2,3,7,8-TCDD of less than 100 fg injected.

The Orbitrap system was able to successfully identify and quantify all PCDD/F congeners in the CS3 standard even in the presence of residues from used engine oil (Figure 2). The presence of the oil residues was clearly demonstrated in the total ion chromatogram of the mixture and was reflected in alterations in the retention times of all PCDD/F peaks. Alterations in retention times of peaks clearly indicated that effects of residues in the used motor oil were at a level that would require additional sample preparation to meet QA criteria. Yet even at this level of contamination the mass spectrometric performance was well with in performance criteria of the method.

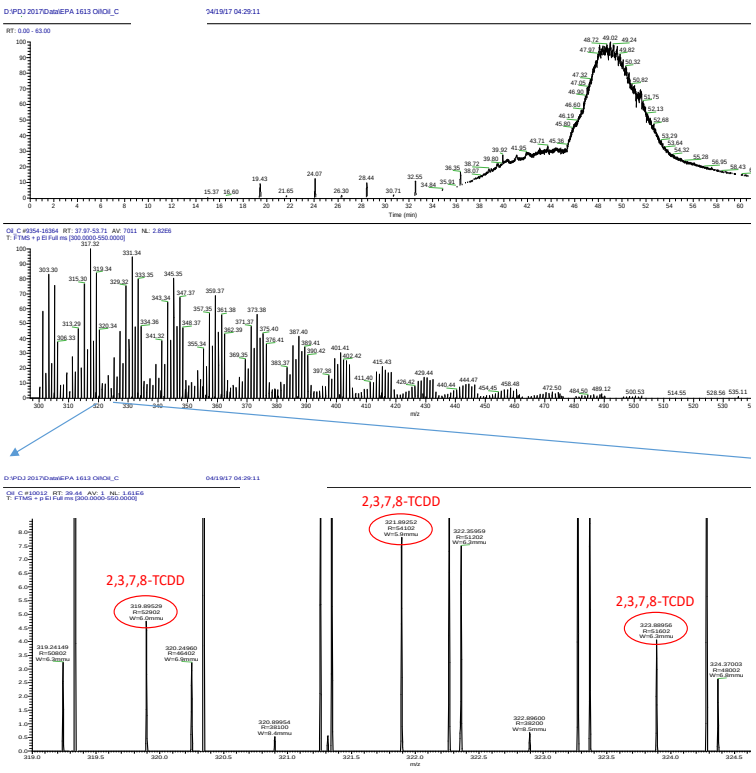

Figure 2 Calibration standard (CS3) mixed with 5 mg of used engine oil extract. Total ion chromatogram (Top) and summed ion profile from 38-54 min. Even in this complex matrix the 3 ions used for 2,3,7,8-TCDD identification can be identified at mass

To further evaluate performance of the QE GC Orbitrap system archived extracts of fish tissue that had previously been analysed on a magnetic instrument were reanalyzed (Table 1). The extracts used for this analysis had been archived at -20°C since 2014 so loss of some congeners or degradation of extract quality was possible. However, results for analysis of a certified reference material (CRM) (WMF-1, Wellington Laboratories, ON) were consistent between the two instruments indicating mimimal degradation of the extracts. The Orbitrap results for the CRM were consistent with the previous analysis in the most part but demonstrated improved quantitation of 2,3,4,7,8-PeCDF and improved quantitation of 2,3,7,8-TCDD and 2,3,7,8-TCDF in one of the three CRM extracts (CRM-3). Improved detection and quantitation of all congeners was also demonstrated in the low level fish extracts that were analyzed. The detection and quantitation of 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HxCDD demonstrated the greatest improvement of the four congeners reported.

Discussion:

The GC-Orbitrap system was capable of meeting the rigorous specificity and sensitivity requirements for identification and quantification of PCDD/Fs as specified in standard methods. Mass resolution of the Orbitrap attained by Fourier Transforms of captured images permitted full-spectrum acquisition compared to the standard single ion monitoring approach commonly applied with magnetic sector systems. This provided enhanced capabilities to detect potential interferences, which increased accuracy and specificity of ion detection. Accuracy of the CG-Orbitrap system was also improved by the comparatively high scan rate, >10 Hz compared to that of systems based on magnetic sector discrimination of masses, where scan rates depend on the number of ions monitored.

Additional advantages of the Orbitrap system are MS/MS capabilities, and in particular the ability to capture full scan MS profile data and MS/MS data simultaneously (data not shown). Collection of MS/MS data provides an additional level of compound verification without impacting calibration or sensitivity. Parameters used to trigger the data dependent MS/MS scans are currently being standardized. The magnetic sector instrument used was a 1992 vintage instrument running OPUS software and so would not be expected to meet accuracy and precision demonstrated by more modern sector instruments. Regardless, data collected on that instrument met all minimum QA/QC criteria of US-EPA method 1613. Similarly, the GC-Orbitrap instrument was able to meet all the method QA/QC criteria and was able to collect additional data (2 confirmation ions, full profile MS data, MS/MS data) to add strength to compound identification. This analysis indicates that the GC-Orbitrap system should be capable of analyzing other semi-volatile residues including polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs) and polychlorinated diphenyl ethers (PCDE), polyhalogenated carbizoles and similar types of compounds or their halogenated analogues.

Table 1 Reanalysis of archived fish sample extracts by Orbitrap GC/MS, initial analysis "AutoSpec" was carried out in 2014. Values less than LOD are in brackets. CRM samples were analyses of the WMF-1 standard reference material. Data for only selected congeners is provided.

	2,3,7,8-TCDD		2,3,7,8-TCDF		1,2,3,6,7,8-HxCDD		2,3,4,7,8_PeCDF	
Sample	Autospec	Orbitrap	Autospec	Orbitrap	Autospec	Orbitrap	Autospec	Orbitrap
CRM-1	10.3	11.4	11.3	14.4	1.1	0.78	2.1	8.92
CRM-2	10.1	11.9	10.1	12.7	(0.2)	(0.1)	1.2	8.20
CRM-3	8.4	10.0	8.8	10.2	(0.7)	1.1	1.1	9.41
Certified Value	13.1±4.4		13.1±4.9		0.88±0.4		7.15±2.2	
FR193	(0.8)	0.88	(0.7)	4.8	(0.3)	3.5 EMPC	(0.3)	3.0
FS147	(0.1)	(0.1)	(1.0)	2.3	(0.7)	2.3	(0.1)	0.6
FR171	(0.8)	(0.1)	(0.6)	0.46	(0.2)	0.5	(0.3)	0.1
FR112	(2.3)	0.16	2.0	2.2	(1.1)	2.2	(0.7)	0.5
FR099	(1.2)	(0.1)	(1.0)	0.32	(0.4)	0.3	(1.0)	0.2
FR151	(1.3)	(0.1)	(1.0)	(0.1)	(0.4)	0.1	(0.4)	(0.1)

References:

EPA Method 1613, Revision B, Tetra- Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, September 15, 1997

Wan Y, Jones PD, Holem RR, Khim JS, Chang H, Kay DP, Roark SA, Newsted JL, Patterson WP, Giesy JP (2010). Bioaccumulation of polychlorinated dibenzo-p-dioxins, dibenzofurans, and dioxin-like polychlorinated biphenyls in fishes from the Tittabawassee and Saginaw Rivers, Michigan, USA. *Sci. Tot. Environ*., 408:2394-2401.