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ESTIMATION OF THE LIMIT OF DETECTION (LOD) FOR POLYCHLORINATED DIBENZO-P-DIOXINS AND FURANS (PCDD/F) AND NON-ORTHO-POLYCHLORINATED BIPHENYLS (CPCBS) ON A THERMO SCIENTIFIC DFS MAGNETIC SECTOR GC-HRMS USING SPLITLESS AND PROGRAMMED TEMPERATURE VAPORIZ

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Introduction

Polychlorinated dibenzo-p-dioxins and furans (PCDD/F) and non-ortho-polychlorinated biphenyls (cPCBs) measurements in human biomonitoring studies have historically been made using gas chromatography isotope dilution high resolution mass spectrometry (GC/ID-HRMS). Over the last decades the serum concentrations of these persistent organic pollutants have been declining in human populations.1 In recent years, the Centers for Disease Control and Prevention has pooled serum for measurements of PCDD/F and cPCBs, to obtain a larger amount of serum and a higher detection frequency for these compounds. In this work, we have used Programmed Temperature Vaporizing (PTV) injector to enable the injection of a larger proportion of the final extract to lower our limit of detection with the objective of enabling individual measurements of PCDD/F and cPCBs in general population serum samples such as the National Health and Nutrition Examination Survey with volumes of approximately 8 grams.

Materials and methods

Standards: We have used the standard (TF-TCDD-MXB; Wellington; ON, Canada) as a sensitivity check standard. This standard contains 1,3,6,8-tetrachlorodibenzo-p-dioxin (1368-TeCDD, 2.0fg/μL), 1379-TeCDD (5.0fg/μL), 1378-TeCDD (10fg/μL), 1478-TeCDD (25fg/μL), 1234-TeCDD (50fg/μL), and 2378-TeCDD (100fg/μL) and the 13C12-labeled 2378-TeCDD (5,000fg/μL). An example chromatogram of the sensitivity check standard is given in Figure 1. LODs estimated according to Taylor2 were assessed using the calibration standard (EDF5524, Cambridge Isotope Laboratories, MA, USA) which contains tetra- through OctaCDD and CDF as well as non-ortho-polychlorinated biphenyls (cPCBs). The calibration range for 2378-TeCDD is 1fg/μL through 10fg/μL and the calibration range for OcCDD is 100fg/μL through 1ng/μL.

Instrumentation: GC/ID-HRMS measurements was conducted on a DFS instrument equipped with a 1310 gas chromatograph and a splitless and PTV injector (Thermo Fisher Scientific). The separations were carried out on a TR-Dioxin-5MS column (length 30m, phase thickness 0.1μm and 0.25mm internal diameter; Thermo Fisher Scientific). The sensitivity check standard was measured using the following GC oven program: 150°C (2.5min) to 200°C (20°C/min) then to 240°C (5°C/min) then to 280°C (20°C/min, hold 3minute). The calibration standard was measured using the following GC program: 150°C (2min) then to 230°C (35°C/min, hold 11min) then to 241°C (1.5°C/min) then to 320°C (20°C/min, hold 2.5min). Splitless injections were made at an injection temperature of 290°C with a constant flow of 0.7mL/minute with a split open time of 1.5 min. PTV injections were made in the splitless mode with at an initial temperature of 140°C (0.5 min) then 10°C/sec to 320°C mL/min (3min).

LOD estimation: The sensitivity check standard was used to compare different instrumental settings for splitless and PTV injections. With this standard we obtained measurements of tetraCDD congeners (N=6) in the concentration range of 2-100fg/μL with one congener at each concentration level. The measured concentrations of each congener in each injection were determined using the 13C12-labeled 2378-TeCDD assuming a slope of one and an intercept of zero. The LOD according to Taylor2 was estimated

from regressing the standard deviation of measured concentration at each concentration against the specified concentration, and defining the LOD as three times the estimated intercept from the regression line (Figure 2 and Table 1).

The LOD for TeCDD was estimated for splitless injection using an injection volume of 1µL of the sensitivity check standard and for the PTV injector the LOD was assessed at injection volumes of 1, 2 and 4µL. For the 4µL injections on the PTV injector the sensitivity check standard was also measured with a 5-fold dilution.

The LOD for all components of the calibration standard was determined based on a data set of six complete calibration curves over two days. An average calibration curve was defined for each day and each calibration point was quantified against the corresponding curve. The LOD was determined using the same approach as above, however the estimated slope and intercept of the calibration curve were used with a LOG10 transformation of the concentration and response variables.

The signal to noise values were based on 4 sigma noise calculated from the Targetquan Quantitation Program (ThermoFisher).

Results and discussion:
The approach used at the CDC for estimating the LOD is based on a statistical approach, published by Taylor2, in which the standard deviation of measured concentrations is estimated as the concentration approaches zero (S0). The S0 is determined by regressing the standard deviation of measured concentration versus specified concentration (Figure 2). The intercept of the resulting regression line corresponds to S0. The LOD is defined as 3 x S0 which corresponds to the point where the relative uncertainty of the measurement is equivalent to ±100%. The benefit of using this approach for assessing the LOD over traditional signal to noise (S/N) determinations is that long-term statistical variability collected over months or even years can be incorporated into the LOD assessment.

The LOD based on Taylor was assessed using splitless injection (1µL) and using a PTV injector operated in the splitless mode (Figure 2AB). As can be seen in Figure 2A we obtained an estimated LOD for TeCDD of 1.3fg/µL for the splitless injector and 1.3, 0.71 and 0.47fg/µL when using the PTV injection of 1, 2 and 4µL, respectively. As expected when expressing the X-axis as amount injected (Figure 2B) we obtain similar estimates of the LOD as 1.5fg injected on-column. The fact that we are estimating similar LODs expressed as injected amount on-column indicates that there is no difference in the amount transferred onto the column regardless of the injection volume and technique used. This finding illustrates that the conditions used for PTV injection are robust and show no measurable losses in the liner, as expected, when operating the PTV injector in the splitless mode. In order to provide comparative data using an S/N-based approach, we also determined the LOD as defined by S/N equal to 3 with the noise factor defined as 4 sigma. The average S/N-based LOD over twenty-two injections was 0.93 fg/µL compared to 0.47 fg/µL for a 4uL injection using the PTV injector.

We obtained a slightly higher LOD of 2fg/µL for 2378-TeCDD with the GC conditions used for the calibration standard and a PTV injection of 4µL. The LODs for the other components of the calibration standard are given in Table 1 and range from 1.1fg/µl (123678-HxCDF) to 190fg/µL (OcCDD). These LODs are comparable or lower than the average estimated concentrations in the NHANES 2005/06 and 2007/08 for the age groups 12-19 and 20-39 years.

We also plotted the isotopic ratio of all measured analytes for the calibration standard and the sensitivity check standard against the specified concentration of each standard as represented for 2378-TeCDD in Figure 3. From these data we could see that the isotopic ratio has a wider distribution at the lower concentrations but typically fall within the specified range of +/- 26%.

By increasing the injection volume on the PTV injector we could lower our detection limit from 1.3fg/µL (splitless, 1µL) to 0.47fg/µL (PTV, splitless mode, 4µL) as determined for the sensitivity check standard. The LOD reported here is the instrumental LOD and does not include interferences from the sample preparation which may raise the LOD. Nevertheless, it is possible to conclude that it may be feasible to measure individual serum samples for studies such as NHANES where 8mL of serum is expected from

each study participant. NHANES is a biannual statistical sampling of the population of the United States and can be used to assess trends in biomonitoring data over time.

References:

1. Aylward, L. L., Hays, S. M. (2002) Journal of Exposure Analysis and Environmental Epidemiology, 12, 319–328.
2. Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC.

Figure 1. Example chromatogram of sensitivity check standard (TF-TCDD-MXB; Wellington; ON, Canada) with a 1µl injection in the splitless mode. The tetrachlorodibenzo-*p*-dioxin (TeCDD) congeners from left to right are: 1368-TeCDD (2 fg/µl); 1379-TeCDD (5 fg/µl); 1378-TeCDD (10 fg/µl); 1478-TeCDD (25 fg/µl); 1234-TeCDD (50 fg/µl); and 2378-TeCDD (100 fg/µl in nonane).

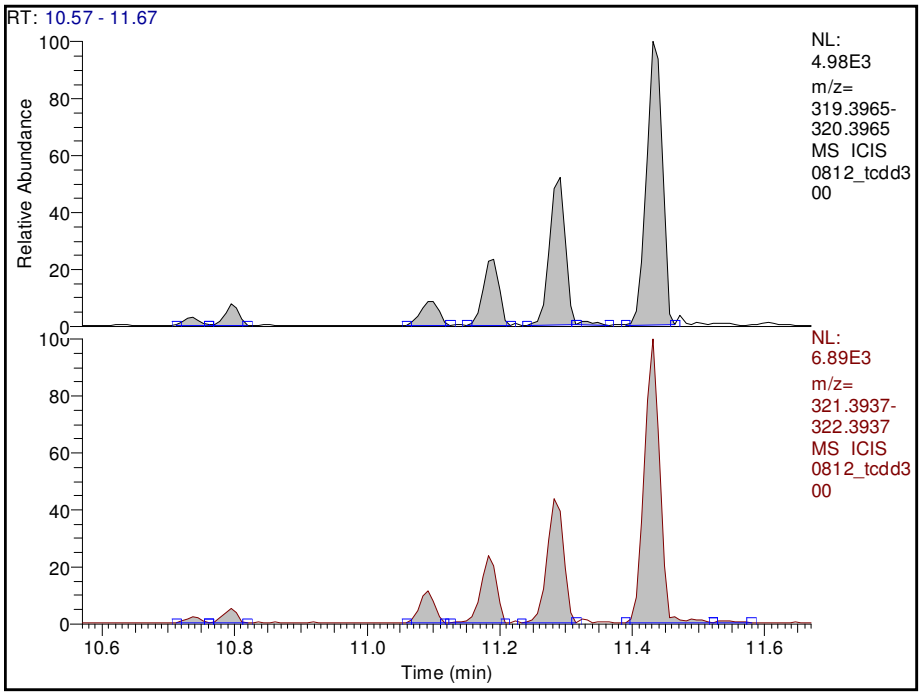

Figure 2. Regression of standard deviation of quantified concentration in method development standard (0.2-100fg/µL and a 5-fold dilution of the standard for splitless injection and Programmed Temperature Vaporization (PTV) injection using 1, 2 and 4µL. The X-axis has been expressed as standard concentration (A) and injected amount on-column (B).

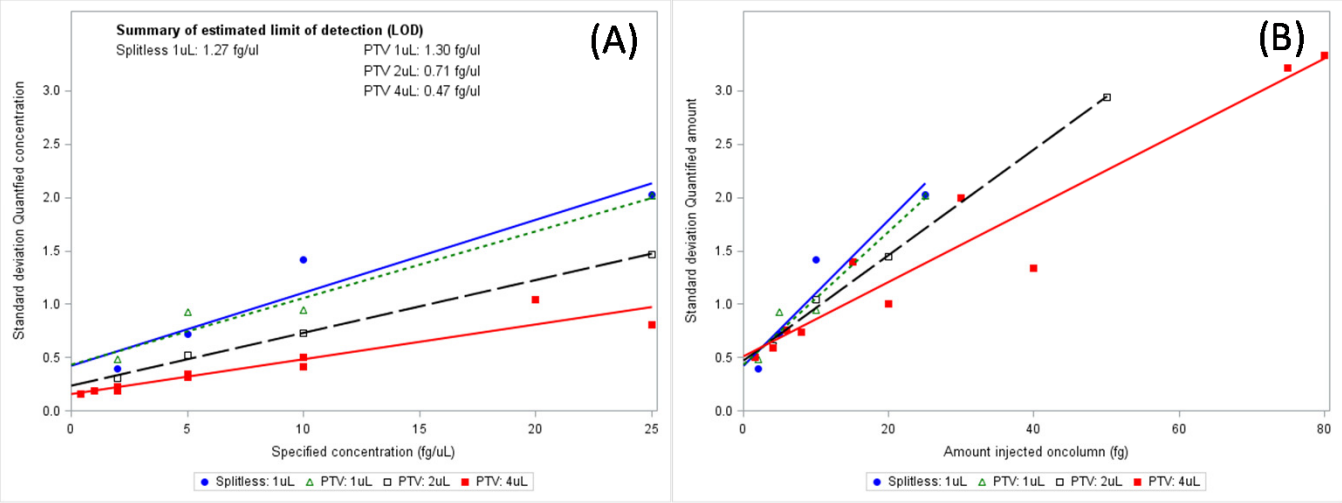

Figure 3. Isotopic ratio of 2378-tetrachlorobibenzo-*p*-dioxin in the calibration standard plotted against specified concentration of standard (1 - 10 fg/μ). The blue line is the theoretical isotopic ratio and the red dashed line is the ±26% tolerance limits.

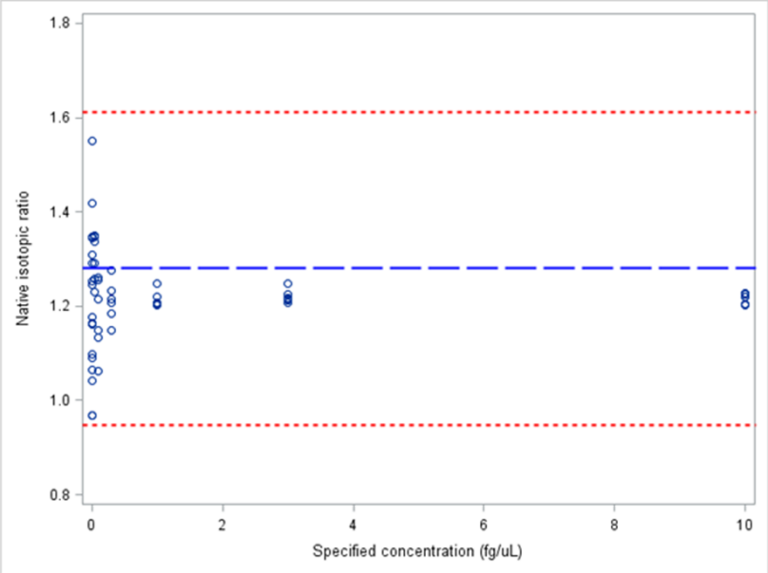

Table 1. Limit of detection (LOD) for PCDD/F and cPCBs in calibration standard. The concentration and detection frequency in the three lowest calibration points are given.

Compound	LOD[1]	Concentration (Detection frequency)			Ratio: Taylor LOD / Cons. CS1
		CS1	CS2	CS3	
Polychlorinated Dioxins (PCDD)					
2378-TeCDD	1.6	1 (83%)	3 (100%)	10 (100%)	1.6
12378-PeCDD	3.0	1 (67%)	3 (100%)	10 (100%)	3.0
123478-HxCDD	3.5	1 (17%)	3 (83%)	10 (100%)	3.5
123678-HxCDD	14	10 (100%)	30 (100%)	100 (100%)	1.4
123789-HxCDD	2.5	1 (33%)	3 (83%)	10 (100%)	2.5
1234678-HpCDD	13	10 (100%)	30 (100%)	100 (100%)	1.3
OcCDD	190	100 (100%)	300 (100%)	1000 (100%)	1.9
Polychlorinated Furans (PCDF)					
2378-TeCDF	1.5	1 (100%)	3 (100%)	10 (100%)	1.5
12378-PeCDF	2.8	1 (100%)	3 (100%)	10 (100%)	2.8
23478-PeCDF	2.0	1 (100%)	3 (100%)	10 (100%)	2.0
123478-HxCDF	1.9	1 (100%)	3 (100%)	10 (100%)	1.9
123678-HxCDF	1.1	1 (50%)	3 (100%)	10 (100%)	1.1
123789-HxCDF	2.2	1 (33%)	3 (83%)	10 (100%)	2.2
234678-HxCDF	2.8	1 (50%)	3 (100%)	10 (100%)	2.8
1234678-HpCDF	22	10 (100%)	30 (100%)	100 (100%)	2.2
1234789-HpCDF	5.1	1 (83%)	3 (100%)	10 (100%)	5.1
OcCDF	2.1	1 (100%)	3 (100%)	10 (100%)	2.1
Non-ortho-PCBs					
PCB77	18	10 (100%)	30 (100%)	100 (100%)	1.8
PCB81	16	10 (100%)	30 (100%)	100 (100%)	1.6
PCB126	19	10 (100%)	30 (100%)	100 (100%)	1.9
PCB169	15	10 (100%)	30 (100%)	100 (100%)	1.5

[1] Limit of Detection according to Taylor.[2]