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AN ASSESSMENT OF AN ORIBITAL TRAPPING MASS SPECTROMETER FOR THE MEASUREMENT OF 2,3,7,8-CHLORINE SUBSTITUTED DIOXINS AND DIBENZOFURANS IN FOODS

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Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans are two classes of related environmental pollutants produced through diverse sources, known to strongly bio-accumulate, and can produce multiple toxic endpoints in animals and are known carcinogens in humans1,2. As such, every practical means is employed to limit exposures and reduce the environmental loadings of these two compound classes. The primary route of non-occupational human exposure to dioxins and furans is foods and the majority coming from animal based foods and often via animal feeds3-5. In the U. S., EPA recently established a reference dose of 0.7 pg/kg body weight per day6. Central to these efforts are confirmatory methods capable of measuring these chemicals are low concentrations in foods.

Materials and methods

Two calibration curves were constructed. One curve made used Wellington Laboratories mixtures of all 17 native 2,3,7,8-substituted congeners and all 17 13C12 labeled standards at concentrations of 0.1, 0.2, 1, 5, 10, 20 and 40 ng/mL. The second used Cambridge Isotope laboratories mixes by diluting EDF-9999-1-5, 10 fold resulting in concentrations of 0.05, 0.2, 0.25, 1, 5, 20 and 100 ng/mL. Test portions of vegetable oil were spiked near the limit of determination for the instrument at the lowest calibrated level (LCL Wellington curve). The vegetable oils were prepared by Pressurized solvent extraction in the presence of acid silica gel followed by acid/basic silica gel and alumina. Sensitivity, mass accuracy and ion ratios were tested using Wellington labs TCDD-MXB and TCDD-MXD (2-100 or 10-1000 fg/µL). The standards or test extracts were measured using a TRACE 1300 GC coupled to QE (Q-Exactive) mass spectrometer (QE-GC) (ThermoFisher, San Jose, CA, USA). GC column was a 40 M DB-5ms with a split/splitless injector(SSL), 4mm ID Restek sky liner. One µL injections were used with the SSL at 300o C for one min splitess. GC programmed from 120o C 2 min. 20o/min. to 200o C 5o/min to 240o C hold 12 min. 10o/min. to 280o C 10 min hold. The QE-GC was operated using default full scan SIM parameters including 70 eV, 50 µamps emission current, E6 target, AGC, mass window for quadrupole set at 240-480 m/z using 281.05114 and 355.06933 lock masses. Transfer line and source temperatures were 250o and 300o C, respectively.

Results and discussion

A gas chromatography quadrupole/orbitrap trap high resolution mass spectrometer (QE-GC) was evaluated for measuring 2,3,7,8-chlorine substituted dibenzo-p-dioxins and dibenzofurans in foods. Figures of merit were mass accuracy <1 ppm for PCDD/Fs at ~50,000 resolving power full width half height (Table 1). Instrumental limit of determination was tested repeatedly using 2,3,7,8-TCDD and other TCDD isomers and was reliably 50 fg with a limit of detection of ≤ 25 fg for TCDD (Table 1 and Figure 1). PeCDD congener required between 75-100 fg to correctly identify this congener (Table 1).

Mass accuracy was always <1ppm with lock mass at all concentrations, isotopic abundances were within 10% at the lowest concentration tested with the exception of PeCDD with the instrument optimized (Table 1). Area counts at approximately 5000 or greater usually gave accurate isotopic abundances (±10%) and quantitation within allowable limits (< ±30%) (Table 1) with notable exception of PeCDD which unfortunately required more response. Figure 1
illustrates one of the many optimized injections of six TCDD isomer mixtures (2-100 fg/µL or 10-1000 fg/µL). Only 4 of the 6 TCDDs at most (2-100 fg/µL) or 5 of 6 (10-1000 fg/µL) were identifiable on most injections depending on daily instrument response. At 10 fg/µL the area response was 50% below the expected based on the response of other TCDD isomers in this and other standards. In addition, the 10 fg/µL TCDD from most injections was not identifiable as TCDD while the 25 fg/µL TCDD isomer level was identified.

Measured amounts were also within allowable limits for all congeners at ½ LCL and the LCL except PeCDD at ½ LCL. A resolution setting of 60,000 FWH was high enough for quantitative resolution of mass interferences from common contamination arising from some PCBs. Nevertheless, quantitatively useful resolution of 1,2,3,7,8-PeCDD from PCB-169 with a common nominal mass at m/z 358 (m/z

357.8511and 357.8439) was not possible at 50,000 resolving power. Area responses for both ions were not near enough to the theoretical predicted isotope abundances. Only at a higher resolving power of 100,000 could a quantitatively useful mass separation be achieved (data not shown).

Table 1. Mean masses (M+2); ∆m; mean isotope ratios (red >15% from theoretical); n=6 standard deviation (sd) of mass error, 0.05 (Tetra/Pentachloro-), 0.1 (Hexa/Heptachloro-) and 0.25 (OCDD and OCDF) ng/mL.

References:

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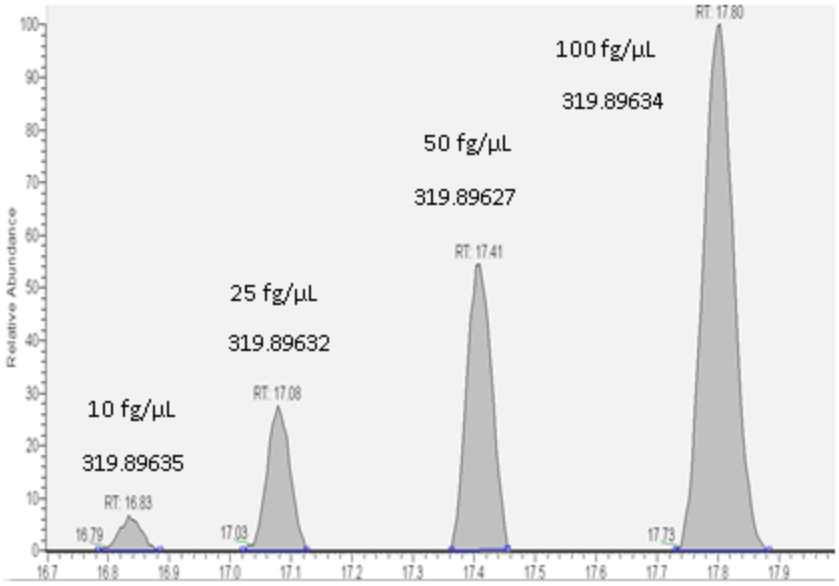
Figure 1. Responses for TCDD isomers from a mixture with concentrations varying from 2-100 fg/µL showing the 4 TCDD peaks from 10-100 fg/µL after ~1.0 µL was injected using auto-sampler mounted syringe. TCDD isomers <10 fg/µL were not identified. Mass resolving power used was 120,000 with no lock mass.

Means	Exact ion (M+2)+	Measured	Δm(sd) ppm	0.05/0.1 Area	Ratio M+2/M+4*	Theor Ratios	$^{13}C_{12}$ Ratio 10ppb
2,3,7,8-TCDD	321.89304	321.89287	0.52(0.31)	11443	0.76	0.764	0.79
1,2,3,7,8-PeCDD	355.85407	355.85394	0.43(0.49)	9551	0.48	0.613	0.63
1,2,3,4,7,8-HxCDD	389.81510	389.81501	0.24(0.31)	18978	1.28	1.224	1.27
1,2,3,6,7,8-HxCDD	389.81510	389.81490	0.5(0.24)	19549	1.26	1.224	1.24
1,2,3,7,8,9-HxCDD	389.81510	389.81499	0.27(0.46)	19193	1.33	1.224	1.26
1,2,3,4,6,7,8-HpCDD	423.77612	423.77641	0.68(0.43)	14561	0.99	1.023	1.04
OCDD	459.73420	459.73430	0.23(0.17)	34543	0.90	0.876	0.9
2,3,7,8-TCDF	305.89813	305.89803	0.33(0.17)	17778	0.78	0.764	0.79
1,2,3,7,8-PeCDF	339.85915	339.85901	0.43(0.25)	16739	1.64	1.53	1.57
2,3,4,7,8-PeCDF	339.85915	339.85898	0.49(0.15)	16227	1.66	1.53	1.56
1,2,3,4,7,8-HxCDF	373.82018	373.81993	0.66(0.33)	29368	1.27	1.224	1.24
1,2,3,6,7,8-HxCDF	373.82018	373.81995	0.63(0.16)	30350	1.26	1.224	1.25
2,3,4,6,7,8-HxCDF	373.82018	373.82002	0.45(0.39)	29551	1.30	1.224	1.25
1,2,3,7,8,9-HxCDF	373.82018	373.82003	0.41(0.23)	24089	1.31	1.224	1.24
1,2,3,4,6,7,8-HpCDF	407.78121	407.78111	0.31(0.23)	26867	1.11	1.023	1.06
1,2,3,4,7,8,9-HpCDF	407.78121	407.78122	0.25(0.11)	15549	1.06	1.023	1.06
OCDF	443.73929	443.73903	0.58(0.22)	44253	0.93	0.876	0.90


Relative Abundance
100 fg/µL
319.89634
RT: 17.80
50 fg/µL
319.89627
RT: 17.41
25 fg/µL
319.89632
RT: 17.08
10 fg/µL
319.89635
RT: 16.83
16.79
17.03
17.73