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MULTIPLE REACTION MONITORING CONDITIONS FOR THE SIMULTANEOUS DETERMINATION OF 2,3,7,8-SUBSTITUTED PCDD/Fs, PCBs 77, 81, 126, 169 AND THE PARALLEL ANALYSIS OF 23 PCBs IN FOODS USING MINI-BORE GAS CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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Introduction

Although food levels have significantly fallen for PCDD/Fs during the past two decades, the demands for isomer specific PCDD/F and PCB determinations have increased. A major focus has been in the area of foods and animal feeds and their components. Three recent animal food contamination incidences have increased the interest in comprehensive monitoring[1-3] of food/feed and feed additives. Recent discoveries of feed additive contamination have sustained an interest in monitoring the most toxic PCDD/F and PCB congeners. FDA has recently greatly expanded the number and type of foods/feeds to be measured annually and will included 26 PCB congeners. The large number of foods, feeds, feed additives that require examination during the course of monitoring programs and contamination events prompted investigators to search for screening techniques. Field-tested screening techniques fall into two categories: bio-analytical assays[4-8] or fast GC(MS)[2,9] with high volume injection[10] and/or coupled to cheap MS techniques[2,10,12-14]. The screening methods require low false negative rates at the action levels while positives and some selected negatives are measured by HRGC/HRMS[3,13]. Tandem mass spectrometry (TMS) in a quadrupole ion trap has been suggested for quick screening for dioxins for several years[11]. Initial reports were limited to TCDD without uniformly labeled TCDD due to software limitations, but produced useful results in three matrices including fish. Recently, a number of reports have suggested useful applications to wastewater, sludge[12], food[13] ash[14], and as an alternative to immunoassay screening[14].

Ion traps produce collision induced dissociation product spectra of stored ions through the application of a waveform(s) across the end-caps matching the periodic motion of stored ion. Increasing the energy of a stored ion can increase the amplitude of the ion motion in the z-axis resulting in either ion ejection as is done in mass axial instability scanning for mass spectrum collection or ion dissociation. Conditions appropriate for each targeted chemical can maximize the formation of product ion with minimal ion loss. The purpose of these studies were to select conditions that optimize a single dissociation channel for PCDDs and PCDFs (M-COCl) and for polychlorobiphenyls (M-Cl_2) and then evaluate these conditions through analysis of first finfish and then other foods.

Methods

Method optimization was performed on a Saturn 2000 quadrupole ion trap equipped with septum programmable on column injection through a Merlin Microseal septumless injector cap. PCDD/Fs and/or PCBs were separated on a 40 M mini-bore (0.18 mm ID). Temperature program for PCDD/Fs was 140 ^{o}C (2 min. hold) to 200 ^{o}C at a rate of 20 ^{o}C/min, then to 240^{o} (12 min hold) at a rate of 5 ^{o}C/mi, and finally to 280 ^{o}C at a rate of 10^{o}/min. PCB temperature program was 120 ^{o}C (2 min hold)

to 200 ^{o}C at a rate of 20 ^{o}C/min to 220 ^{o}C (10 min. hold) at a rate of 2 ^{o}C/min, and finally to 290 ^{o}C at a rate of 10^{o}/min.

PCB product ion spectra were adjusted to the highest response while maximizing the spectral difference between mon-ortho chlorine substituted congeners and di-ortho chlorine substituted congeners. Twenty-six instrument parameters for PCDD/F and PCBs are optimized through tests with all targeted analytes[15]. The final parameter conditions for the 44 congeners are evaluated with a five point standard curve and initially through the analysis finfish species collected in Lake Chardara, Kazakhstan and bluefish, Atlantic salmon, and rockfish obtained commercially in Washington, DC USA[16]. Test portions of finfish were fortified with 100 pg $^{13}C_{12}$-labeled PCDD/Fs, 250 pg $^{13}C_{12}$-labeled PCBs 77, 126, 169 and 1000 pg $^{13}C_{12}$-PCBs 28, 52, 101, 105, 114, 118, 138, 153, 156, 157, 167 180, 189. Fish samples were prepared as previously described[17] except the PCB containing fractions were saved and clean up over a multi-layer K^{+}silicate/sulfuric acid silica gel/neutral silica gel column followed with Florisil or a neutral alumina column.

Results and Discussion

Conditions selected for PCDD/Fs and PCBs 77, 81, 126, and 169 are listed in Table 1. PCDD/Fs all use M+1 for the precursor ion, except for HpCDD/Fs and OCDD/F (M+3), while PCB precursor selection utilizes M+2. PCDD/Fs are dissociated using a narrow band of frequencies (5-9) so that the stored precursor ions, M+ and M+2, are completely dissociated. The resulting product ion spectrum contains product ions with chlorine isotopic responses that are of equal or nearly equal intensity when part of the loss contains a single chlorine atom (M-COCl, M-Cl, M-CO_2Cl etc). Instrumental LODs for TCDD are approximately 100 fg for either product ion using these GC conditions[15]. PCDDs and PCDFs were standardized from 0.4 – 200 pg or 4-1000 pg for PCBs 77, 126 and 169 with an average RSD for all 20 congeners of 6%. The standard curve for the remaining PCBs between 5-5000 pg gave an average RSD of 11% for all congeners. LODs for PCBs 77, 126 and 169 were found to be similar to the PCDD/Fs. Repeat analysis of bluefish, rockfish and salmon for the detected PCDD/Fs and PCBs 77,126 and 169 gave RSDs below the 20% method criteria. Figure 1 shows the product ion spectra for a mon-ortho PCB and di-ortho PCB dissociated using a single frequency at 2V excitation amplitude. Notice that the presence of a PCB-99 (di-ortho-pentachloro congener) is easier to detect due to a larger response from M-Cl when excitation energy is set at 2V rather than at 4V and is nearly absent from PCB 118 under all excitation energies. Because mono-ortho congeners have greater dioxin-like toxicity, it is important to distinguish them from potentially co-eluting di-ortho congeners.

Conclusions

Product ion response for PCBs were higher using a single frequency during excitation rather than a narrow band as is used for the PCDD/Fs. PCDD/F conditions produced high sensitivity while also monitoring non-ortho-PCBs. Because the selected product ion loss for dioxin-like PCBs is the loss of 2 chlorine atoms, no advantage is found by fragmenting precursor ions other than M+2 (or M+4). The spectral differences found with the lower excitation energies provide a high degree of selectivity, while maintaining sufficient sensitivity. The spectral differences also allow a constant check on chromatographic resolution of closely eluting mono-ortho and di-ortho congeners, thereby reducing or eliminating the need for extensive separations before the GC/MS.

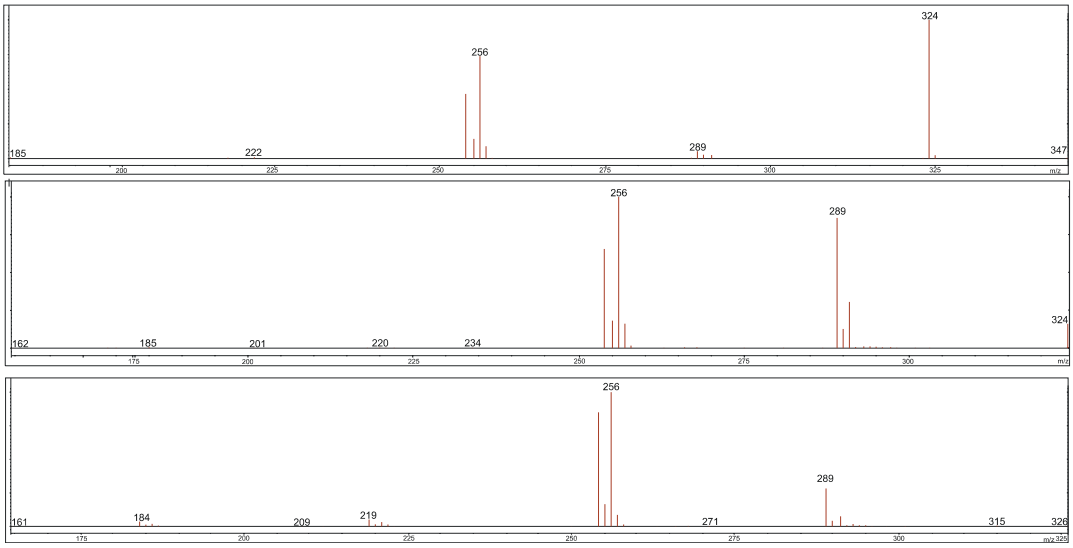

Figure 1: Product spectra; PCB-118, PCB-99 at 2V top/middle respectively; PCB-99 (bottom) at 4V.

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Table 1: Quadrupole ion storage tandem mass spectrometry (QISTMS) conditions[1]; PCDD/F and PCB acquisition segments in elution order on a DB-5 ms capillary column.

Segment (#)/ Congener	**Precursor ion (m/z)**	**LMCO (m/z)**	**CID (V)**	**CID freq. #**	**Product ion m/z**	TIR[2]**(Obs.)**
(1) PCB 77+81	292	129	2.0	1	220,222	1.0**(1.04**)
$^{13}C_{12}$	304	135	2.0	1	232,234	
(2)TCDF	305	135	3.5	9	241,243	1.37**(1.6)**
$^{13}C_{12}$	317	140	3.4	9	252, 254	
$^{13}C_{12}$-TCDD	334	148	3.0	9	268, 270	
(3) TCDD	321	141	2.8	9	257, 259	1.37**(1.4)**
$^{13}C_{12}$	336	148	3.0	9	268, 270	
PCB-126	326	144	2.25	1	254, 256	0.66**(0.64)**
$^{13}C_{12}$-PCB 126					266, 268	
(4) PeCDF	339	151	3.8	9	275, 277	1.03**(1.15)**
$^{13}C_{12}$	351	156	3.8	9	286, 288	
PeCDD	355	155	2.0	5	291, 293	1.03**(1.07)**
$^{13}C_{12}$	367	162	2.0	5	302, 304	
PCB-169	360	159	2.5	1	288, 290	0.5**(0.49)**
$^{13}C_{12}$-PCB-169	372	164	2.5	1	300, 302	
(5) HxCDF	373	166	4.0	9	309, 311	0.82**(0.84)**
$^{13}C_{12}$	385	171	4.0	9	320, 322	
HxCDD	389	171	3.0	13	325, 327	0.82**(0.86)**
$^{13}C_{12}$	401	178	3.0	13	336, 338	
(6) HpCDF	409	181	3.6	13	345, 347	1.86**(2.03)**
$^{13}C_{12}$	421	187	3.5	13	356, 358	
HpCDD	425	187	2.0	5	361, 363	1.86**(1.69)**
$^{13}C_{12}$	435	193	2.0	5	370, 372	
(7) OCDF	444	194	4.0	9	379, 381	1.53**(1.4)**
OCDD	460	201	3.5	9	395, 397	1.53**(1.6)**
$^{13}C_{12}$	471	209	3.3	9	406, 408	

[1]CID time (ms) = 10 (PCBs, 4 ms), mass window = 3 m/z (334=1 m/z), maximum ion time = 65 msec, emission current = 100 μamps, scan rate = 0.27 sec, multiplier offset = +300 volts, target RIC = 5,000. LMCO = low-mass cut off (CID). Mass window $^{13}C_{12}$-2,3,7,8-TCDD = 5 and 2 for PCBs and low and high mass offsets set to 2 DACs. [2]TIR(Obs.) = theoretical isotope ratio(observed).