Determination of Polychlorinated Dibenzo-p-dioxin and Dibenzofuran Background in Milk and Cheese by Quadrupole Ion Storage Collision-Induced Dissociation MS/MS

Douglas G. Hayward
Methods Research Branch, Division of Pesticides and Industrial Chemicals, US Food and Drug Administration, 200 C St., SW, Washington, DC 20204

Abstract

Recent developments in quadrupole ion storage scanning techniques have made possible the acquisition of mass spectrometry/mass spectrometry (MS/MS) data with high selectivity and reproducibility. Retail dairy products were analyzed for bioincurred or background contamination by all 17 of the 2,3,7,8-polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Analytes were measured by both full-scan electron ionization low resolution MS (EI-LRMS) and collision-induced dissociation (CID) MS/MS. Results were comparable for both techniques. However, MS/MS provided higher sensitivity and selectivity for many congeners. Results for the CID MS/MS technique were reproducible, with little reduction in sensitivity or spectral quality during the analyses of all test samples. MS/MS data acquisition was initiated after calibration using 2-150 pg standard and three or four selected ions in each MS/MS spectrum. The responses were linear for all ions calibrated. The MS/MS signal-to-noise ratio (S/N) was 10 to 100 times greater than that for EI-LRMS performed with the same instrument. S/N increased for most parent ions as well as for all daughter ions. Further development of this technique may provide a cost-effective alternative to traditional high resolution MS analyses for PCDDs and PCDFs in food.

Introduction

The generally accepted major source of exposure to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) is food.[1] The levels of international toxic equivalents (I-TEQ) found in human foods, other than fish, are usually 1 ng/kg or lower on a fat-adjusted basis.[2-7] Accurate and selective analysis of foods for all PCDDs and PCDFs requires a dedicated high resolution mass spectrometer operating close to optimum manufacturer's specifications. Analyses may also require large test portions, depending on the day-to-day instrument performance, the levels anticipated in the food and the desired quantitation limits. Even with adequate equipment and human resources, the analyses are time consuming and costly.

Quadrupole ion storage mass spectrometry (MS) has provided an inexpensive, highly sensitive alternative for obtaining full-scan spectral data for numerous organic compounds including dioxins. In this laboratory, full-scan spectral data have been generated with as little as 2 pg of tetrachlorodibenzo-p-dioxin/tetrachlorodibenzofuran (TCDD/F) and 20 pg of octachlorodibenzo-p-dioxin/octachlorodibenzofuran (OCDD/F). However, these quantitation limits could not be duplicated with food extracts. The high detection limits and low resolution spectra (poor selectivity) routinely obtainable with food extracts severely limit this approach to PCDD and PCDF determinations in foods.

High specificity for PCDD/Fs has been demonstrated by using MS/MS,[8-10] but high sensitivity for PCDD/Fs in foods is not easily obtained at a reasonable cost. Most instruments that generate MS/MS data are as costly to purchase and operate as high resolution instruments and are 10 to 50 times less sensitive.[9] Development of scanning techniques that apply a high frequency supplemental dipole field to the ion trap endcaps, matching the resonant frequency of a selectively stored ion, allows collection of MS/MS data. When an ion trap detector with MS/MS capability was used, low (sub parts-per-trillion) levels of background or of bioincurred and spiked PCDDs and PCDFs were successfully measured in milk and cheese. Optimization of the MS/MS technique is discussed.

Experimental

Sample collection

Milk and cheese were collected from Washington, DC, area retail stores and an additional sample was provided by the Florida Department of Agriculture and Consumer Services. Commercially packaged milk was promptly frozen and thawed before analysis; cheese was stored at 4°C.

Extraction and cleanup

Test portions were fortified with 15 $^{13}C_{12}$-labeled PCDD and PCDF congeners ($^{13}C_{12}$-labeled OCDF and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HxCDD) were not added) at 1 ng/kg wet weight (cheese was fortified at 5 ng/kg). In addition, duplicate test portions were fortified with 15 native congeners (all 2,3,7,8-substituted congeners except OCDD and OCDF) at either 0.192 or 0.96 ng/kg wet weight (cheese was fortified at 1.0 ng/kg).

Milks (200 g) were extracted and cleaned up as previously described by using a modified AOAC extraction procedure[11] and a modified method based on the Smith and Stalling procedure.[12] After the addition of 2000 pg octachloronaphthalene in nonane, the extracts were evaporated to 5 μL. Octachloronaphthalene served as the recovery standard and an internal standard for quantitation in the MS/MS experiments.

MS/MS and EI-LRMS calibration and analyte quantitation

MS/MS and EI-LRMS spectra were generated by using a Varian Saturn 3 gas chromatograph/mass spectrometer (quadrupole ion storage

mass spectrometer running version 5.0 software, fitted with a waveboard containing chemical ionization and MS/MS options). Several instrument parameters were optimized before any calibration was attempted, including: (1) storage rf voltage, (2) collision-induced dissociation (CID) bandwidth, (3) mass isolation window, (4) excitation energy, (5) modulation range and (6) collision gas pressure. Collision gas pressure has recently been demonstrated to be an important factor in optimizing the production of daughter ions in quadrupole storage MS/MS.[13] However, this parameter was kept at He pressures in the optimum range developed for EI-LRMS. All other parameters were optimized separately for each homolog group.

Calibration data for all 17 of the 2,3,7,8-substituted congeners were acquired for 2-150 pg of each congener on column by using a septum-programmable on-column injector. Data were recorded and stored for three or four selected ions in the MS/MS daughter ion spectrum of each congener. Response factors relative to octachloronaphthalene were calculated. Nearly all selected masses show good linearity (Table 1). Individual congener results (Table 2) are an average of values calculated from intensities of all calibrated ions measured. The final result was also corrected for the recovery of the $^{13}C_{12}$-labeled congener.

Results and Discussion

The MS/MS mode achieved a higher signal-to-noise ratio (S/N) than was obtained by the EI-LRMS mode in analyses of milk and cheese. The near absence of background signals in the reconstructed daughter ion chromatograms was indicative of the high selectivity. MS/MS quantitation limits were estimated on the basis of the spike responses and measurement of the confirmed bioincurred or background contaminants. The lowest quantitation limits were found for 2,3,7,8-TCDF and 2,3,7,8-TCDD (0.5-1 pg). All pentachlorodibenzo-p-dioxin/pentachlorodibenzofuran (PeCDD/F) and hexachlorodibenzo-p-dioxin/hexachlorodibenzofuran (HxCDD/F) congeners had quantitation limits of 2 pg. Heptachlorinated congeners did not fragment as well and, therefore, had higher quantitation limits (3-5 pg). OCDD and OCDF were less sensitive mainly because of low fragmentation and chromatography. The quantitation limits for OCDD and OCDF were 5-10 pg. At these quantitation limits parent ion was still measurable for all congeners except PeCDDs and HxCDDs.

Contaminants in milk and cheese were identified and quantitated in the pg/kg range (cheese data are not shown in Table 2). Results for unfortified milk were similar to those reported for milk collected in rural areas with no known sources of contamination.[5] The I-TEQ of the Washington, DC, milk sample was slightly higher than the mean found by Furst et al.[4] for 120 milk samples in Germany. The I-TEQ for the Florida milk was twice that of the Washington, DC, milk, but about the same as that for the unspiked cheese. This difference was most likely attributable to variations in laboratory background rather than to significant differences in bioincurred levels.

The analysis of milk fortified at 0.19 ng/kg gave levels of analytes that were 10 to 15% higher than the amounts added.

Table 1. Mean response factors relative to octachloronaphthalene and relative standard deviations for TCDD/Fs (2, 2, 15 pg), PeCDD/Fs, HxCDD/Fs and HpCDD/Fs (10, 25, 75 pg) and OCDD/Fs (20, 50, 150 pg)

Analyte	m/z	Mean RRF	RSD, %		m/z	Mean RRF	RSD, %
2,3,7,8-TCDF	245	0.23	22	1,2,3,7,8,9-HxCDF	313	0.25	9
2,3,7,8-TCDF	243	0.56	9	1,2,3,7,8,9-HxCDF	311	0.33	5
2,3,7,8-TCDF	304	0.96	10	1,2,3,7,8,9-HxCDF	374	0.48	4
2,3,7,8-TCDF	306	0.59	11	1,2,3,7,8,9-HxCDF	376	0.18	11
2,3,7,8-TCDD	261	0.15	7	1,2,3,4,7,8-HxCDD	329	0.37	6
2,3,7,8-TCDD	259	0.60	9	1,2,3,4,7,8-HxCDD	327	0.79	7
2,3,7,8-TCDD	320	1.00	22	1,2,3,4,7,8-HxCDD	388	0.49	14
2,3,7,8-TCDD	322	0.25	9	1,2,3,6,7,8-HxCDD	329	0.29	12
1,2,3,7,8-PeCDF	279	0.06	11	1,2,3,6,7,8-HxCDD	327	0.61	11
1,2,3,7,8-PeCDF	277	0.37	4	1,2,3,6,7,8-HxCDD	388	0.37	5
1,2,3,7,8-PeCDF	338	0.62	13	1,2,3,7,8,9-HxCDD	329	0.67	11
1,2,3,7,8-PeCDF	340	0.58	9	1,2,3,7,8,9-HxCDD	327	0.31	7
2,3,4,7,8-PeCDF	279	0.20	9	1,2,3,7,8,9-HxCDD	388	0.45	7
2,3,4,7,8-PeCDF	277	0.37	8	1,2,3,4,6,7,8-HpCDF	349	0.12	1
2,3,4,7,8-PeCDF	338	0.69	9	1,2,3,4,6,7,8-HpCDF	347	0.16	4
2,3,4,7,8-PeCDF	340	0.65	4	1,2,3,4,6,7,8-HpCDF	408	0.81	7
1,2,3,7,8-PeCDD	295	0.27	2	1,2,3,4,6,7,8-HpCDF	410	0.63	6
1,2,3,7,8-PeCDD	293	0.75	8	1,2,3,4,6,7,8-HpCDD	361	0.38	8
1,2,3,7,8-PeCDD	354	0.70	15	1,2,3,4,6,7,8-HpCDD	363	0.36	5
1,2,3,4,7,8-HxCDF	313	0.22	15	1,2,3,4,6,7,8-HpCDD	424	0.33	10
1,2,3,4,7,8-HxCDF	311	0.26	5	1,2,3,4,7,8,9-HpCDF	349	0.15	7
1,2,3,4,7,8-HxCDF	374	0.72	5	1,2,3,4,7,8,9-HpCDF	347	0.25	8
1,2,3,4,7,8-HxCDF	376	0.36	7	1,2,3,4,7,8,9-HpCDF	408	0.78	12
1,2,3,6,7,8-HxCDF	313	0.24	19	1,2,3,4,7,8,9-HpCDF	410	0.51	3
1,2,3,6,7,8-HxCDF	311	0.26	5	OCDD	399	0.16	16
1,2,3,6,7,8-HxCDF	374	0.75	6	OCDD	397	0.42	10
1,2,3,6,7,8-HxCDF	376	0.37	4	OCDD	458	0.67	5
2,3,4,6,7,8-HxCDF	313	0.20	12	OCDD	460	0.15	3
2,3,4,6,7,8-HxCDF	311	0.23	9	OCDF	383	0.22	7
2,3,4,6,7,8 HxCDF	374	0.72	6	OCDF	381	0.13	3
2,3,4,6,7,8 HxCDF	376	0.39	4	OCDF	442	0.57	8
				OCDF	444	0.68	8

Mean = average of three calibration points (e.g., 2, 5, 15 pg TCDD); one injection at each level.

Abbreviations: RRF = relative response factor; RSD = relative standard deviation; TCDF, PeCDF, HxCDF, HpCDF and OCDF = tetrachloro-, pentachloro-, hexachloro-, heptachloro- and octachlorodibenzofuran, respectively; TCDD, PeCDD, HxCDD, HpCDD and OCDD = tetrachloro-, pentachloro-, hexachloro-, heptachloro- and octachlorodibenzo-p-dioxin, respectively.

Table 2. CID MS/MS determinations of PCDDs and PCDFs in cow's milk, ng/kg wet weight; Florida milk spiked at 0.19 ng/kg and Washington, DC, milk spiked at 0.96 ng/kg; OCDD and OCDF not spiked

Analyte	Florida milk, unfortified		Florida milk, fortified		Wash., DC, milk, unfortified		Wash., DC, milk, fortified
	ng/kg						
2,3,7,8-TCDD	0.019	B	0.198		0.006	*	0.85
1,2,3,7,8-PeCDD	0.042	L	0.273		0.0097	*	1.03
1,2,3,4,7,8-HxCDD	0.039	L	0.215		0.029	*	0.97
1,2,3,6,7,8-HxCDD	0.147	L	0.276		0.076	B	1.29
1,2,3,7,8,9-HxCDD	0.086	B	0.214		0.023	*	0.87
1,2,3,4,6,7,8-HpCDD	0.204	L	0.401	L	0.21		0.81
1,2,3,4,6,7,8,9-OCDD	0.697	L	0.63	L	0.34	L	ND
2,3,7,8-TCDF	0.032	B	0.222		0.006	*	1.1
1,2,3,7,8-PeCDF	0.075	B	0.229		0.007	*	1.19
2,3,4,7,8-PeCDF	0.051	L	0.249		0.023	L	1.03
1,2,3,4,7,8-HxCDF	0.061	L	0.249		0.023	L	0.98
1,2,3,6,7,8-HxCDF	0.052	L	0.241		0.02	L	1.07
1,2,3,7,8,9-HxCDF	ND		0.172		0.035	L	1.03
2,3,4,6,7,8-HxCDF	0.049	L	0.241		0.059	L	1.02
1,2,3,4,6,7,8-HpCDF	0.131	L	0.298	L	0.087	L	1.1
1,2,3,4,7,8,9-HpCDF	0.055	B	0.216		0.059	L	1.07
1,2,3,4,6,7,8,9-OCDF	0.18	L	0.112	L	0.063	*	ND
I-TEQ	0.12		0.66		0.05		2.80

All values were corrected for recovery. See abbreviations defined in Table 1.
ND = Not determined. * = Less than MDL. B = Less than QL.
L = Upper limit; analyte detected in blank.
MDL = 10 times the noise level for all ions; QL = 3 times the MDL.
I-TEQ = International toxic equivalent.
Spiking was done with a standard lot different from that used for MS/MS calibration.

Comparison with results from analysis of unfortified milk suggests that elevated recoveries are due to both bioincurred residues and/or laboratory background (Table 2). Results from analysis of fortified (1 ng/kg) and unfortified cheese were comparable when measured by both full-scan MS and MS/MS when the residue levels were high. Low pg/kg levels of residues were identified and measured by MS/MS and not by EI-LRMS.

Conclusions

There are clear avenues for improving the sensitivity of the MS/MS technique. Sensitivity can be improved by further optimizing MS conditions during ion formation in ways not possible under the full-scan MS mode. Further optimization of emission current and the electron multiplier and collision gas pressure should be investigated. In this study, ions stored with

q_z values between 0.3 and 0.5 gave the highest summed intensity of parent and daughter ion. These findings are consistent with CID MS/MS experiments on other aromatic compounds in the ion trap.[13] All MS/MS data acquisition was performed by using a value of q_z = 0.3 for all PCDDs and PCDFs. However, further optimization may be possible.

Mention of certain vendors in this manuscript does not imply endorsement of any product by the US Food and Drug Administration.

References

1. Hattemer-Frey, HA, and Travis, CC (1989): Comparison of human exposure to dioxin from municipal waste incineration and background environmental contamination. Chemosphere 18:643-649.
2. Beck, H, Eckart, K, Mathar, W, and Wittkowski, R (1989): PCDD and PCDF body burden from food intake in the Federal Republic of Germany. Chemosphere 18:417-424.
3. Furst, P, Furst, C, and Groebel, W (1990): Levels of PCDDs and PCDFs in food-stuffs from the Federal Republic of Germany. Chemosphere 20:787-792.
4. Furst, P, Furst, C, and Wilmers, K (1992): Survey of dairy products for PCDDs, PCDFs, PCBs and HCB. Chemosphere 25:1039-1048.
5. Startin, JR, Rose, M, Wright, C, Parker, I, and Gilbert, J (1990): Surveillance of British foods for PCDDs and PCDFs. Chemosphere 20:793-798.
6. Theelen, RMC, Liem, AKD, Slob, W, and Van Wijnen, JH (1993): Intake of 2,3,7,8 chlorine substituted dioxins, furans and planar PCBs from food in the Netherlands: median and distribution. Chemosphere 27(9):1625-1635.
7. Schecter, A, Startin, J, Wright, C, Kelly, M, Papke, O, Lis, A, Ball, M, and Olson, J (1994): Dioxins in U.S. food and estimated daily intake. Chemosphere 29:2261-2265.
8. Tondeur, Y, Niederhut, WN, and Campana, JE (1987): A hybrid HRGC/MS/MS method for the characterization of tetrachlorinated-p-dioxins in environmental samples. Biomed. Environ. Mass Spectrom. 14:449-456.
9. Charles, MJ, Green, B, Tondeur, Y, and Hass, JR (1989): Optimization of a hybrid-mass spectrometer method for the analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. Chemosphere 19:51-57.
10. Charles, MJ, and Tondeur, Y (1990): Choosing between high-resolution mass spectroscopy and mass spectroscopy/mass spectroscopy: environmental applications. Environ. Sci. Technol. 24:1856-1860.
11. Official Methods of Analysis (1990) 15th Ed., Association of Official Analytical Chemists, Arlington, VA, sec. 970.52L.
12. Smith, LM, Stalling, DL, and Johnson, JL (1984): Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Anal. Chem. 56:1830-1842.
13. Charles, MJ, Mcluckey, SA, and Glish, GL (1994): Competition between resonance ejection and ion dissociation during resonant excitiation in a quadrupole ion trap. J. Am. Soc. Mass. Spectrom. 5:1031-1041.